

Air Quality Criteria for Ozone and Related Photochemical Oxidants (Second External Review Draft)

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
U.S. Environmental Protection Agency
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DISCLAIMER

This document is a second external review draft for review purposes only and does not constitute U.S. Environmental Protection Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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 (O₃) in 1979 and a 1-hour O₃ NAAQS was set. The 1978 document focused mainly on the air quality criteria for O₃ and, to a lesser extent, on those for other photochemical oxidants (e.g., hydrogen peroxide and the peroxyacyl nitrates), as have subsequent revised versions of the ozone document.

To meet Clean Air Act requirements noted above for periodic review of criteria and NAAQS, the O₃ criteria document, *Air Quality Criteria for Ozone and Other Photochemical Oxidants*, was next revised and then 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 some newer scientific data that became available after completion of the 1986 criteria document. Such literature was assessed in the next periodic revision of the O₃ air quality criteria document (completed in 1996) and provided scientific bases supporting the setting by EPA in 1997 of an 8-h O₃ NAAQS that is currently in force together with the 1-h O₃ standard.

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 Ozone Air Quality Criteria Document (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, some other scientific data are also presented and evaluated 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 or accepted for publication through 2004.

The present Second Draft O₃ AQCD (dated August 2005) is being released for public comment and review by the Clean Air Scientific Advisory Committee (CASAC) to obtain comments on the organization and structure of the document, the issues addressed, the approaches employed in assessing and interpreting the newly available information on O₃ exposures and effects, and the key findings and conclusions arrived at as a consequence of this assessment. Public comments and recommendations will be taken into account making any appropriate further revisions to this document for incorporation into the final version of the document to be completed and issued by February 28, 2006. Evaluations contained in the present document will be drawn on to provide inputs to associated PM 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. The document also evaluates ambient O₃ environmental effects on vegetation and ecosystems, man-made materials, and surface level solar UV radiation flux and global climate change, 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 draft document.

**Air Quality Criteria for Ozone and Related
Photochemical Oxidants
(Second External Review Draft)**

VOLUME I

Executive Summary	E-1
1. INTRODUCTION	1-1
2. PHYSICS AND CHEMISTRY OF OZONE IN THE ATMOSPHERE	2-1
3. ENVIRONMENTAL CONCENTRATIONS, PATTERNS, AND EXPOSURE ESTIMATES	3-1
4. DOSIMETRY, SPECIES HOMOLOGY, SENSITIVITY, AND ANIMAL-TO-HUMAN EXTRAPOLATION	4-1
5. TOXICOLOGICAL EFFECTS OF OZONE AND RELATED PHOTOCHEMICAL OXIDANTS IN LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS	5-1
6. CONTROLLED HUMAN EXPOSURE STUDIES OF OZONE AND RELATED PHOTOCHEMICAL OXIDANTS	6-1
7. EPIDEMIOLOGICAL STUDIES OF HUMAN HEALTH EFFECTS ASSOCIATED WITH AMBIENT OZONE EXPOSURE	7-1
8. INTEGRATIVE SYNTHESIS: EXPOSURE AND HEALTH EFFECTS	8-1
9. ENVIRONMENTAL EFFECTS: OZONE EFFECTS ON VEGETATION AND ECOSYSTEMS	9-1
10. TROPOSPHERIC OZONE EFFECTS ON UV-B FLUX AND CLIMATE CHANGE PROCESSES	10-1
11. EFFECT OF OZONE ON MAN-MADE MATERIALS	11-1

**Air Quality Criteria for Ozone and Related
Photochemical Oxidants
(Second External Review Draft)**

(cont'd)

VOLUME II

CHAPTER 2 ANNEX (ATMOSPHERIC PHYSICS/CHEMISTRY)	AX2-1
CHAPTER 3 ANNEX (AIR QUALITY AND EXPOSURE)	AX3-1
CHAPTER 4 ANNEX (DOSIMETRY)	AX4-1
CHAPTER 5 ANNEX (ANIMAL TOXICOLOGY)	AX5-1
CHAPTER 6 ANNEX (CONTROLLED HUMAN EXPOSURE)	AX6-1
CHAPTER 7 ANNEX (EPIDEMIOLOGY)	AX7-1

VOLUME III

CHAPTER 9 ANNEX (ENVIRONMENTAL EFFECTS)	AX9-1
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Table of Contents
(cont'd)

		<u>Page</u>
AX9.2.4	Wounding and Pathogen Attack	AX9-75
	AX9.2.4.1 Peroxidases	AX9-77
	AX9.2.4.2 Jasmonic Acid and Salicylic Acid	AX9-79
	AX9.2.4.3 Stress-Induced Alterations in Gene Expression	AX9-81
AX9.2.5	Primary Assimilation by Photosynthesis	AX9-84
	AX9.2.5.1 Photooxidation: Light Reactions	AX9-84
AX9.2.6	Alteration of Rubisco by Ozone: Dark Reactions	AX9-85
AX9.2.7	Carbohydrate Transformations and Translocation	AX9-88
	AX9.2.7.1 Lipid Synthesis	AX9-90
AX9.2.8	Role of Age and Size Influencing Response to Ozone ...	AX9-93
AX9.2.9	Summary	AX9-95
REFERENCES	AX9-97
AX9.3	MODIFICATION OF FUNCTIONAL AND GROWTH RESPONSES	AX9-106
AX9.3.1	Introduction	AX9-106
AX9.3.2	Genetics	AX9-109
	AX9.3.2.1 Genetic Basis of Ozone Sensitivity ...	AX9-110
AX9.3.3	Environmental Biological Factors	AX9-112
	AX9.3.3.1 Oxidant-Plant-Insect Interactions	AX9-113
	AX9.3.3.2 Oxidant-Plant-Pathogen Interactions ..	AX9-116
	AX9.3.3.3 Oxidant-Plant-Symbiont Interactions ..	AX9-121
	AX9.3.3.4 Oxidant-Plant-Plant Interactions: Competition	AX9-122
AX9.3.4	Physical Factors	AX9-124
	AX9.3.4.1 Light	AX9-125
	AX9.3.4.2 Temperature	AX9-128
	AX9.3.4.3 Humidity and Surface Wetness	AX9-132
	AX9.3.4.4 Drought and Salinity	AX9-133
AX9.3.5	Nutritional Factors	AX9-137
AX9.3.6	Interactions with Other Pollutants	AX9-139
	AX9.3.6.1 Oxidant Mixtures	AX9-140
	AX9.3.6.2 Sulfur Dioxide	AX9-140
	AX9.3.6.3 Nitrogen Oxides, Nitric Acid Vapor, and Ammonia	AX9-140
	AX9.3.6.4 Hydrogen Fluoride and Other Gaseous Pollutants	AX9-143
	AX9.3.6.5 Acidic Deposition	AX9-144
	AX9.3.6.6 Heavy Metals	AX9-145
	AX9.3.6.7 Mixtures of Ozone with Two or More Pollutants	AX9-145

Table of Contents
(cont'd)

		<u>Page</u>
AX9.3.7	Interactions with Agricultural Chemicals	AX9-146
AX9.3.8	Factors Associated with Global Climate Change	AX9-147
	AX9.3.8.1 Ozone-Carbon Dioxide-Temperature Interactions	AX9-148
	AX9.3.8.2 Ozone-UV-B Interactions	AX9-171
	AX9.3.8.3 Interactions of Ozone with Multiple Climate Change Factors	AX9-173
AX9.3.9	Summary - Environmental Factors	AX9-174
REFERENCES	AX9-178
AX9.4	EFFECTS-BASED AIR QUALITY EXPOSURE INDICES	AX9-196
AX9.4.1	Introduction	AX9-196
AX9.4.2	Summary of Conclusions from the Previous Criteria Document	AX9-197
AX9.4.3	Evaluation of Various Exposure Indices for Describing Ambient Exposure-Response Relationships	AX9-199
AX9.4.4	Identifying Exposure Components That Relate to Vegetation Effects	AX9-205
	AX9.4.4.1 Role of Concentration	AX9-205
	AX9.4.4.2 Role of Duration	AX9-208
	AX9.4.4.3 Patterns of Exposure	AX9-209
	AX9.4.4.4 Frequency of Occurrence of Peak Concentrations	AX9-213
	AX9.4.4.5 Canopy Structure	AX9-213
	AX9.4.4.6 Site and Climate Factors	AX9-214
	AX9.4.4.7 Plant Defense Mechanism - Detoxification	AX9-214
AX9.4.5	Ozone Uptake or Effective Dose as an Index	AX9-215
	AX9.4.5.1 Models of Stomatal Conductance	AX9-216
	AX9.4.5.2 Nonlinear Response and Developing Flux Indices	AX9-219
	AX9.4.5.3 Simulation Models	AX9-221
AX9.4.6	Summary	AX9-221
REFERENCES	AX9-224
AX9.5	OZONE EXPOSURE-PLANT RESPONSE RELATIONSHIPS	AX9-230
AX9.5.1	Introduction	AX9-230
AX9.5.2	Summary of Key Findings/Conclusions from Previous Criteria Documents	AX9-231
AX9.5.3	Ozone Indices and Ambient Exposure	AX9-241
AX9.5.4	Effects of Ozone on Annual and Biennial Species	AX9-260
	AX9.5.4.1 Effects on Growth, Biomass, and Yield of Individual Species	AX9-261

Table of Contents

(cont'd)

		<u>Page</u>
	AX9.5.4.2 Effects on Plant Quality	AX9-267
	AX9.5.4.3 Effects on Foliar Symptoms	AX9-268
	AX9.5.4.4 Other Effects	AX9-269
	AX9.5.4.5 Scaling Experimental Data to Field Conditions	AX9-270
	AX9.5.4.6 Summary of Effects on Short- Lived Species	AX9-273
AX9.5.5	Effects of Ozone on Long-Lived (Perennial) Species ...	AX9-275
	AX9.5.5.1 Herbaceous Perennial Species	AX9-275
	AX9.5.5.2 Deciduous Woody Species	AX9-280
	AX9.5.5.3 European Critical Levels	AX9-287
	AX9.5.5.4 Summary of Effects on Deciduous Woody Species	AX9-287
	AX9.5.5.5 Evergreen Woody Species	AX9-288
	AX9.5.5.6 Summary of Effects on Evergreen Woody Species	AX9-292
	AX9.5.5.7 Scaling Experimental Data to Mature Trees	AX9-292
AX9.5.6	Studies with the Chemical EDU	AX9-296
AX9.5.7	Summary	AX9-298
REFERENCES	AX9-302
AX9.6	EFFECTS OF OZONE EXPOSURE ON NATURAL ECOSYSTEMS	AX9-313
AX9.6.1	Introduction	AX9-313
AX9.6.2	Case Studies	AX9-321
	AX9.6.2.1 Valley of Mexico	AX9-321
	AX9.6.2.2 San Bernardino Mountains	AX9-322
	AX9.6.2.3 Sierra Nevada Mountains	AX9-325
	AX9.6.2.4 Appalachian Mountains	AX9-330
	AX9.6.2.5 <i>Plantago</i> Studies in the United Kingdom	AX9-331
	AX9.6.2.6 Forest Health in the Carpathian Mountains	AX9-331
	AX9.6.2.7 Field Exposure System (FACE), Rhinelander, Wisconsin	AX9-333
AX9.6.3	Landscape Condition	AX9-333
AX9.6.4	Biotic Condition	AX9-337
	AX9.6.4.1 Ecosystems and Communities	AX9-337
	AX9.6.4.2 Species and Populations	AX9-343
AX9.6.5	Organism Condition	AX9-347
AX9.6.6	Ecosystem, Chemical, and Physical Characteristics (water, soil)	AX9-355

Table of Contents
(cont'd)

	<u>Page</u>	
AX9.6.6.1	Nutrient Concentrations, Trace Inorganic and Organic Chemicals	AX9-355
AX9.6.7	Ecological Processes	AX9-355
AX9.6.7.1	Energy Flow	AX9-355
AX9.6.7.2	Material Flow	AX9-358
AX9.6.8	Hydrological and Geomorphological	AX9-361
AX9.6.9	Natural Disturbance Regimes	AX9-361
AX9.6.10	Scaling to Ecosystem Levels	AX9-362
AX9.6.10.1	Scaling from Seedlings to Mature Trees	AX9-363
AX9.6.10.2	Surveys, Growth Correlations and Stand-Level Modeling	AX9-365
AX9.6.11	Summary of Ecological Effects of Ozone Exposure on Natural Ecosystems	AX9-372
REFERENCES		AX9-376
AX9.7	ECONOMIC EVALUATION OF OZONE EFFECTS ON AGRICULTURE, FORESTRY AND NATURAL ECOSYSTEMS	AX9-394
AX9.7.1	Introduction	AX9-394
AX9.7.2	The Measurement of Economic Information	AX9-395
AX9.7.3	Understanding of Air Pollutants Effects on the Economic Valuation of Agriculture and Other Vegetation in the 1996 Criteria Document	AX9-397
AX9.7.3.1	Agriculture	AX9-398
AX9.7.3.2	Forests (Tree Species) and Natural Ecosystems	AX9-400
AX9.7.4	Studies Since 1996 of Ozone Exposure Effects on the Economic Value of Agriculture, Forests, and Ecosystems	AX9-402
AX9.7.5	Limitations of Scientific Studies and Economic Information	AX9-404
AX9.7.6	Conclusions	AX9-407
REFERENCES		AX9-408

List of Tables

<u>Number</u>		<u>Page</u>
AX9-1	Advantages and Disadvantages of Protective Chemicals Used in Assessment of O ₃ Effects on Plants	AX9-19
AX9-2	Advantages and Disadvantages of Bioindicators Used to Study O ₃ Plant Effects	AX9-20
AX9-3	Advantages and Disadvantages of Cultivar Comparisons Used in Assessment of O ₃ Effects on Plants	AX9-24
AX9-4	Advantages and Disadvantages of Various Dendrochronological Techniques Used in Assessment of O ₃ Effects on Plants	AX9-26
AX9-5	Advantages and Disadvantages of Modeling Techniques Used in Assessment of O ₃ Effects on Plants	AX9-29
AX9-6	The Flow of Ozone into a Leaf and Possible Reactions	AX9-50
AX9-7	Some Rates of Reaction of Ozone with Critical Biochemicals	AX9-55
AX9-8	Superoxide Dismutase Isozymes and Isoforms	AX9-72
AX9-9	Gene Families and cDNA Clones Used as Probes for SAR (Ward et al., 1991)	AX9-76
AX9-10	Proteins Altered by Ozone as Measured by Molecular Biological Techniques as mRNA Level or Other Gene Activity Rather than Enzyme Activity	AX9-82
AX9-11	Interactions Involving O ₃ and Plant Pathogens	AX9-117
AX9-12	Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels	AX9-150
AX9-13	Summary of Ozone Exposure Indices Calculated for 3- or 5-Month Growing Seasons from 1982 to 1991	AX9-233
AX9-14	Ozone Exposure Levels (Using Various Indices) Estimated to Cause at Least 10% Crop Loss in 50 and 75% of Experimental Cases	AX9-235
AX9-15	SUM06 Levels Associated with 10 and 20% Total Biomass Loss for 50 and 75% of the Seedling Studies	AX9-238
AX9-16	Summary of Selected Studies of Ozone Effects on Annual Species	AX9-243

List of Tables
(cont'd)

<u>Number</u>		<u>Page</u>
AX9-17	Summary of Selected Studies of the Effects of Ozone on Perennial Herbaceous Plants	AX9-247
AX9-18	Summary of Selected Studies of Ozone Effects on Deciduous Trees and Shrubs	AX9-249
AX9-19	Summary of Selected Studies of Ozone Effects on Evergreen Trees and Shrubs	AX9-254
AX9-20	Ethylene Diurea Effects on Vegetation Responses to Ozone	AX9-257
AX9-21	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	AX9-275
AX9-22	Essential Ecological Attributes for Natural Ecosystems Affected by O ₃	AX9-315
AX9-23	Case Studies Demonstrating the Ecological Effects of O ₃	AX9-324
AX9-24	The Most Comprehensively Studied Effects of O ₃ on Natural Ecosystem are for the San Bernardino Mountain Forest Ecosystem	AX9-326
AX9-25	Effects of Ozone, Ozone and N Deposition, and Ozone and Drought Stress on <i>Pinus ponderosa</i> and <i>Pinus jeffreyi</i> in the Sierra Nevada and the San Bernardino Mountains, California	AX9-327
AX9-26	Summary of Responses of <i>Populus tremuloides</i> 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	AX9-334

List of Figures

<u>Number</u>		<u>Page</u>
AX9-1	Ozone uptake from the atmosphere	AX9-40
AX9-2	Absorption and transformation of O ₃ within the leaf	AX9-42
AX9-3	The uptake of O ₃ into the leaf	AX9-47
AX9-4	The microarchitecture of a dicot leaf	AX9-47
AX9-5	The change in the O ₃ concentration inside a leaf with time	AX9-49
AX9-6	Possible transformations of O ₃ within a leaf	AX9-51
AX9-7	Possible reactions of O ₃ within water	AX9-52
AX9-8a,b	The Crigee mechanism of O ₃ attack of a double bond	AX9-54
AX9-9	Varied ESR radicals, trapped and not, generated by O ₃ under somewhat physiological conditions	AX9-57
AX9-10	Pathogen-Induced Hypersensitivity	AX9-60
AX9-11	The interaction of H ₂ O ₂ and Ca ²⁺ movements with ABA-induced stomatal closure	AX9-63
AX9-12	The reaction of ascorbate within the apoplasm of the cell wall and its ultimate reduction/oxidations	AX9-67
AX9-13	The pathway leading from phospholipids to jasmonic and traumatic acid	AX9-80
AX9-14	The production of Rubisco and its Calvin Cycle pathway reactions	AX9-86
AX9-15	Linkage of senescence with hypersensitivity reactions and the first event of O ₃ attack of plants	AX9-93
AX9-16	Diagrammatic representation of several exposure indices, illustrating how they weight concentration and accumulate exposure	AX9-200
AX9-17	Trends in May-September 12-h SUM06, peak 1-h ozone concentration and number of daily exceedances of 95 ppb for Crestline in 1963-1999 in relation to trends in mean daily maximum temperature for Crestline and daily reactive organic gases (ROG) and oxides of nitrogen (NO _x) for San Bernardino county	AX9-207

List of Figures
(cont'd)

<u>Number</u>		<u>Page</u>
AX9-18	The number of hourly average concentrations between 50 and 89 ppb for the period 1980 to 2000 for the Crestline, San Bernardino, CA monitoring site	AX9-209
AX9-19	Distribution of biomass loss predictions from Weibull and linear exposure-response models that relate biomass to O ₃ exposure	AX9-237
AX9-20	A conceptual diagram of processes and storage pools in sources and sinks that are affected by O ₃ exposure	AX9-335
AX9-21	Common anthropogenic stressors and the essential ecological attributes they affect	AX9-373

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Abbreviations and Acronyms

ABA	abscisic acid
ABI ₂	phospho-tyrosine-specific protein phosphatase
ACC	1-aminocyclopropane-1-carboxylate
ACS	1-aminocyclopropane-1-carboxylase synthase
A _{max}	maximum photosynthesis rate
ANN	artificial neural network
ANOVA	analysis of variance
ANP	Acadia National Park
AOS	allene oxide synthase
AOT40	seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb
AOT60	seasonal sum of the difference between an hourly concentration at the threshold value of 60 ppb, minus the threshold value of 60 ppb
AOT _x	cumulative, cutoff concentration-based exposure index
APX	ascorbate peroxidase
AQCD	Air Quality Criteria Document
A _{sat}	photosynthetic assimilation in saturating light
AVG	1-aminoethoxyvinyl-glycine
AZO	azoxystrobin
CEC	controlled environment chambers
CF	charcoal-filtered
CFA	charcoal/Purafil-filtered air
CFI	continuous forest inventory
CHIP	Effects of Elevated Carbon Dioxide and Ozone on Potato Tuber Quality in the European Multiple Site Experiment
CO ₂	carbon dioxide
CSTRs	continuous stirred tank reactors
CU	cumulative uptake

cyt	cytochrome
DG	diacylglycerol
DGDG	digalactosyldiacylglycerol
DHA	dehydroascorbate
DMPO	dimethylphrrrolise 1-oxide; 5,5-dimethyl-1-pyrroline N-oxide
DNA	deoxyribonucleic acid
ECM	ectomycorrhizal fungi
EDU	ethylenediurea
EEAs	essential ecological attributes
EMEP	European Monitoring and Evaluation Program
EPA	U.S. Environmental Protection Agency
EPO	epoxyconazole
EPR	electron paramagnetic resonance; ESR
ESPACE-wheat	European Stress Physiology and Climate Experiment on the Effects of Carbon Dioxide and Oxygen on Spring Wheat
ESR	electron spin resonance; EPR
ET	ethylene
EU	European Union
FA	fatty acid
FACE	free-air carbon dioxide enrichment (system)
FFAs	free fatty acids
FHM	Forest Health Monitoring (assessment)
FLAG	Federal Land Managers' Air Quality Related Values Workgroup
FPM	Forest Pest Management
GHG	greenhouse gas
GR	glutathione reductase
GRSM	Great Smoky Mountains National Park
GSH	glutathione
GSH-Px	glutathione peroxidase

GSSG	glutathione disulfide
H ⁺	hydrogen ion
2HDM, 2ndHDM	second-highest daily maximum 1-h concentration
HNO ₃	nitric acid
H ₂ O ₂	hydrogen peroxide
HO ₃ •	protonated ozone radical
HO•	hydroxyl radical
HO ₂ •	hydroperoxyl; hydroperoxy radical
HPOT	13-hydroperoxide linolenic acid
HR	hypersensitive response
IPCC	Intergovernmental Panel on Climate Change
JA	jasmonic acid
J _{max}	maximum rate of electron transport for the regeneration of RuBP
J _{sat}	saturating light
KROFEX	Krauzberg Ozone Fumigation Experiment
LAI	leaf area index
mAOT	modified accumulated exposure over the threshold
MDA	malonaldehyde
MDGD	monogalactosyldiacylglycerol
mRNA	messenger ribonucleic acid
MV	methyl viologen
NAD ⁺	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADP ⁺	nicotinamide adenine dinucleotide phosphate
NADPH, NAD(P)H	reduced nicotinamide adenine dinucleotide phosphate
NaE	sodium erythorbate
NCLAN	National Crop Loss Assessment Network
n.d.	no data

NDF	neutral detergent fiber
NF	national forest
NF	non-filtered
NH ₃	ammonia
(NH ₄) ₂ SO ₄	ammonium sulfate
N ₂ O	nitrous oxide
NO	nitric oxide
NO ₂	nitrogen dioxide
NO ₃ ⁻	nitrate
NO _x	nitrogen oxides
NP	national park
NPP	net primary productivity
n.s.	nonsignificant
O ₂ ⁻	superoxide
O ₂ ^{-•}	superoxide radical
O ₃	ozone
OD	outer diameter
OTC	open-top chamber
p, P	probability value
PAD	pollutant applied dose
PAL	phenylalanine lyase
PAN	peroxyacetyl nitrate
PAR	photosynthetically active radiation
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PFD	photosynthetic flux density
PG	phosphatidyl glycerol
PGSW	Plant Growth Stress Model

PI	phosphatidyl inositol
ppb	parts per billion
ppm	parts per million
PQH ₂	plastoquinone
PR	pathogenesis-related (protein)
PSII	Photosystem II; ϕ PSII
qP	photochemical quenching
r ²	correlation coefficient
R ²	multiple regression correlation coefficient
<i>rbcL</i>	Rubisco large subunit
<i>rbcS</i>	Rubisco small subunit
RH	relative humidity
RNA	ribonucleic acid
ROG	reactive organic gases
ROS	reactive oxygen species
Rubisco	ribulose-1,6-P ₂ -carboxylase/oxygenase1
RuBP	ribulose biphosphate
SA	salicylic acid
SAB	Science Advisory Board
SAR	systemic acquired resistance
SD	standard deviation
SE	standard error
SHEN	Shenandoah National Park
SLA	specific leaf area
SMD	soil moisture deficit
SNAAQs	Secondary National Ambient Air Quality Standards
SO ₂	sulfur dioxide
SO ₄ ²⁻	sulfate

SOD	superoxide dismutase
SUM00	sum of all hourly average concentrations
SUM06	seasonal sum of all hourly average concentrations ≥ 0.06 ppm
SUM08	seasonal sum of all hourly average concentrations ≥ 0.08 ppm
TMPO	tetramethylpyrrolidone 1-oxide; 3,3,5,5-dimethyl-1-pyrrolidine N-oxide
TNC	total nonstructural carbohydrate
UDGT	UDP galactose-1,2,-diacylglycerol
UNECE	United Nations Economic Commission for Europe
UNECE ICP-Vegetation	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)
UNEP	United Nations Environment Program
UV-B	ultraviolet radiation of wavelengths from 280 to 320 nm
VOC	volatile organic compound
VPD	vapor pressure deficit
W126	cumulative integrated exposure index with a sigmoidal weighting function
ZAPS	Zonal Air Pollution System

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 O₃ 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 O₃, or
- (2) provided information about the extent and geographic distribution of the responses of herbaceous and woody plants, both cultivated and native, to ambient O₃ 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 O₃; it usually involves treating plants to a range of artificial O₃ 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 O₃ 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 O₃, are not discussed here but are addressed in Section AX9.2. Methods used to characterize the O₃ content of ambient air and to define exposure and exposure-response relations are discussed in Sections AX9.4 and AX9.5, respectively.

1 The methodologies for exposure-response studies have involved many different types of
2 exposure facilities and protocols and have employed a range of statistical approaches in the
3 analysis and interpretation of the data. Most of the studies have been conducted using major
4 agricultural crop species. The methodologies have improved over the years as a result of the
5 development, availability, or application of new or improved instrumentation, physical systems,
6 and numerical approaches to data analysis. Yet equally important to the roles played by these
7 advances has been the clearer understanding that has emerged from earlier work identifying the
8 *type* of experimentation needed to achieve realistic assessments of the magnitude and extent of
9 the impact of O₃ on vegetation of all types. As a result, significantly increased attention is now
10 being paid to field observations and biomonitoring, particularly to the responses of forest trees
11 and native vegetation.

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

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

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

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

29 The earliest experimental investigations of the effects of O₃ on plants utilized simple glass
30 or plastic-covered chambers, often located within greenhouses, into which a flow of O₃-enriched
31 air or oxygen could be passed to provide the exposure. The types, shapes, styles, materials of

1 construction, and locations of these chambers were as numerous as the different investigators
2 and, in spite of providing little resemblance to real-world conditions, they yielded much of the
3 basic information on the visible and physiological effects on plants. The construction and
4 performance of more elaborate and better instrumented chambers dating back to the 1960s has
5 been well-summarized in Hogsett et al. (1987a), including those installed in greenhouses (with
6 or without some control of temperature and light intensity).

7 One greenhouse chamber approach that continues to yield useful information on the
8 relationships of O₃ uptake to both physiological and growth effects employs continuous stirred
9 tank reactors (CSTRs) first described by Heck et al. (1978). Although originally developed to
10 permit mass-balance studies of O₃ flux to plants, their use has more recently widened to include
11 short-term physiological and growth studies of O₃ × CO₂ interactions (e.g., Costa et al., 2001;
12 Heagle et al., 1994b; Loats and Rebbeck, 1999; Rao et al., 1995; Reinert and Ho, 1995; and
13 Reinert et al., 1997), and of surveys of native plant responses to O₃ (Orendovici et al., 2003).
14 In many cases, supplementary lighting and temperature control of the surrounding structure have
15 been used to control or modify the environmental conditions (e.g., Heagle et al., 1994a).

16 Many investigations have utilized commercially available controlled environment
17 chambers and walk-in rooms adapted to permit the introduction of a flow of O₃ into the
18 controlled air-volume. Such chambers continue to find use in genetic screening and in
19 physiological and biochemical studies aimed primarily at improving our understanding of modes
20 of action. For example, some of the ongoing studies of the O₃ responses of *Plantago major*
21 populations have been conducted in controlled environment chambers (Reiling and Davison,
22 1994; Whitfield et al., 1996b).

23 The environmental conditions provided by indoor chambers of any type will always
24 preclude the use of the information obtained with such chambers in predicting O₃ effects in the
25 natural environment, because the environmental conditions will always be measurably different
26 from field conditions. However, highly sophisticated controlled environment chambers such as
27 those described by Langebartels et al. (1997), which are subdivided into aerial and root
28 compartments with dynamic control of light intensity and photoperiod, air and soil temperature,
29 humidity, soil moisture, wind speed, and exposure to O₃, may come close to simulating specific
30 natural conditions. Such chambers have provided meaningful insights into a wide array of the
31 early biochemical responses of plants to O₃. They can also minimize confounding factors that

1 make indoor chamber studies only rarely able to be extrapolated to field conditions, e.g., that
2 shoots and roots develop under different temperature regimes. The applicability of the results
3 of many chamber studies may be further limited by their use of container-grown plants. Most
4 of the concerns over the applicability of CO₂ enrichment studies, as discussed in Section
5 AX9.5.7.1, may also be relevant to O₃ enrichment studies, as suggested by Whitfield et al.
6 (1996a).

7 Whitfield et al. (1996a) reported significant interactive effects between O₃ and soil volume
8 on the growth of *Plantago major*. They noted that although container size may limit root and,
9 hence, plant growth, the reverse may also be true for single plants in large containers, which do
10 not experience typical field competition for resources. Other studies found little or no effect of
11 rooting volume on plant response to O₃. Heagle et al. (1979a,b; 1983) found that four wheat
12 cultivars (*Triticum aestivum* L.) had similar proportional suppression of seed yield to season-
13 long O₃ exposure whether plants were grown in the ground or in 3.8-L pots. Similarly,
14 proportional O₃ injury and yield response of field corn (*Zea mays* L.) (Heagle et al., 1979a) and
15 soybean (*Glycine max* (L.) Merr.) (Heagle et al., 1983) was similar whether the plants were
16 grown in 15-L pots or in the ground. In a two-year experiment with soybean, the relative effects
17 of CO₂ and O₃ on above-ground biomass and seed yield were similar whether the plants were
18 grown in pots (15 and 21 L) or grown in the ground (Booker et al., 2005). Collectively, the
19 results suggest that while planting density and rooting environment affect plant morphology and
20 growth, the relative responses of seed yield to elevated O₃ may be similar whether plants are
21 grown in pots or in the ground.

22 23 **AX9.1.2.2 Field Chambers**

24 Although some types of closed field chambers have largely fallen out of favor in recent
25 years, closed “Solardome” field chambers (Lucas et al., 1987; Rafarel and Ashenden, 1991) have
26 been successfully used in studies of O₃ × acid mist interactions (Ashenden et al., 1995, 1996).

27 Concern over the need to establish realistic plant-litter-soil relationships as a prerequisite to
28 studies of the effects of O₃ and CO₂ enrichment on ponderosa pine (*Pinus ponderosa*) seedlings
29 led Tingey et al. (1996) to develop closed, partially environmentally controlled, sun-lit chambers
30 (“terracosms”) incorporating 1-m-deep lysimeters containing forest soil in which the appropriate
31 horizon structure was retained.

1 In general, field chamber studies are dominated by the use of various versions of the open-
2 top chamber (OTC) design, first described by Heagle et al. (1973) and Mandl et al. (1973). Most
3 chambers are ~3 m in diameter with 2.5-m-high walls. Hogsett et al. (1987a) described in detail
4 many of the various modifications to the original OTC designs that appeared subsequently, e.g.,
5 the use of larger chambers to permit exposing small trees (Kats et al., 1985) and grapevines
6 (Mandl et al., 1989), the addition of a conical baffle at the top to improve ventilation (Kats et al.,
7 1976), a frustrum at the top to reduce ambient air incursions, and a plastic rain-cap to exclude
8 precipitation (Hogsett et al., 1985). All of these modifications included the discharge of air via
9 ports in annular ducting or interiorly perforated double-layered walls at the base of the chambers
10 to provide turbulent mixing and the upward mass flow of air.

11 Wiltshire et al. (1992) described a large OTC suitable for small trees with roll-up sides that
12 permitted the trees to be readily subjected from time to time to episodic, normal, “chamberless”
13 environmental conditions. In the 6-m-high OTCs described by Seufert and Arndt (1985) used
14 with Norway spruce (*Picea abies*) trees, a second zone of annular enrichment was also provided
15 between 4 and 5 m. The use of OTCs was adopted for the large European Stress Physiology and
16 Climate Experiment on the effects of CO₂ and O₃ on spring wheat (ESPACE-wheat), conducted
17 over 1994 to 1996 at field sites in eight countries (Jäger et al., 1999). However, typical
18 European chambers have the introduction of O₃-enriched air at or above canopy height. The
19 relatively low costs of fabrication, operation, and maintenance has favored OTC use in field
20 studies (Fangmeier et al., 1992; Musselman and Hale, 1997). The air supplied to the chambers
21 can be readily filtered through activated charcoal to reduce the O₃ concentration, or it can be
22 enriched with O₃ to provide a range of exposures.

23 All field chambers create internal environments that differ from ambient air, giving rise to
24 so-called “chamber effects” with the modification of microclimatic variables, including reduced
25 and uneven light intensity, uneven rainfall, constant wind speed, reduced dew formation, and
26 increased air temperatures (Fuhrer, 1994; Manning and Krupa, 1992). Because of the constant
27 wind speed and delivery systems, OTCs can provide a more definable exposure than free-air
28 systems can due to the lack of “hot-spots”, where exposures are essentially undefined, in free-air
29 systems. Nonetheless, there are several characteristics of the OTC design and operation that can
30 lead to unrealistic exposures. First, the plants are subjected to constant turbulence, which,
31 through increased uptake resulting from the consequently low boundary layer resistance to

1 diffusion, may lead to overestimation of cause-effect relationships (Krupa et al., 1995; Legge
2 et al., 1995). However, in at least one case where canopy resistances were quantified in OTCs
3 and in the field, it was determined that gaseous pollutant exposure to crops in OTCs was similar
4 to that which would have occurred at the same concentration in the field (Unsworth et al., 1984a,
5 1984b).

6 A second concern is that the introduction of the O₃-enriched air into the lower part of
7 chambers as described by Heagle et al. (1973) and Mandl et al. (1973) results in a O₃
8 concentration gradient that decreases with increasing height, the converse of the situation
9 observed in ambient air in which the O₃ concentration decreases markedly from above a plant
10 canopy to ground level (Grünhage and Jäger, 1994; Pleijel et al., 1995, 1996). Concern that
11 studies conducted in such OTCs may somewhat overestimate the effects of O₃ led to the
12 European design, which provides a decreasing downward gradient. It seems unlikely that the
13 “chamber effects” produced by the two designs will be the same. These issues are discussed
14 more fully in Section AX9.1.2.4.

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

20 The difficulties faced in the experimental exposure of forest trees to air pollutants in
21 chambers (e.g., Seufert and Arndt [1985]) led to the development of branch chambers such as
22 those described by Ennis et al. (1990), Houpis et al. (1991), and Teskey et al. (1991). These
23 chambers are essentially large cuvettes without temperature control and, as noted by Musselman
24 and Hale (1997), share many of the characteristics of CSTRs, i.e., transparent walls, internal
25 fans, and inlet and outlet monitors to permit the determination of O₃ uptake, CO₂ exchange, and
26 transpiration. Although they make it possible to expose whole branches to different O₃ regimes,
27 the relevance of the data they yield in regard to the whole tree may be questionable. As noted by
28 Saxe et al. (1998), the inevitable change in environmental conditions resulting from the isolation
29 of the branch may cause different responses from those that would be obtained if the whole tree
30 had been subjected to the same environmental conditions.

31

1 **AX9.1.2.3 Plume Systems**

2 Plume systems are chamberless exposure facilities in which the atmosphere surrounding
3 plants in the field is modified by the injection of pollutant gas into the air above or around them
4 from multiple orifices spaced to permit diffusion and turbulence so as to establish relatively
5 homogeneous conditions as the individual plumes disperse and mix with the ambient air. As
6 pointed out by Manning and Krupa (1992), they can only be used to *increase* the O₃ levels in the
7 ambient air. The volume of air to be modified is unconfined, and three approaches have been
8 used to achieve desired pollutant concentrations in the air passing over the plants, producing
9 various systems that

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

13 Gradient systems created by dispensing a pollutant gas into the air at canopy level from
14 perforated horizontal pipes arranged at right angles to the prevailing wind were described
15 for SO₂ studies in the early 1980s. A modified gradient system for O₃ was used by Bytnerowicz
16 et al. (1988) to study effects on desert species, but there appear to have been no recent
17 applications of the method. A gradient O₃-*exclusion* system is discussed in Section AX9.2.3.1.

18 Systems designed to achieve spatially uniform pollutant levels by ensuring that the release
19 of a pollutant is always on the upwind side of the study site were also originally described
20 for SO₂ studies (e.g., Greenwood et al. [1982]). However, the adaptation of these concepts as
21 introduced by McLeod et al. (1985) in constructing a large circular field site for exposing crops
22 to SO₂ led to the subsequent development of both the large-scale O₃ and SO₂ fumigation system
23 for forest trees in the United Kingdom in 1985 (the Liphook Forest Fumigation Project) (1992),
24 the smaller system for O₃ fumigation constructed at Kuopio, Finland in 1990 (Wulff et al.,
25 1992), and the free-air carbon-dioxide enrichment (FACE) systems of gas dispersal over crops
26 (Hendrey and Kimball, 1994) and forest trees (Hendrey et al., 1999). Although originally
27 designed to provide chamberless field facilities for studying the CO₂ effects of climate change,
28 large forest tree FACE systems have recently been adapted to include the dispensing of O₃

1 (Karnosky et al., 1999). Volk et al. (2003) recently described a system for exposing grasslands
2 that uses 7-m diameter plots. FACE systems discharge the pollutant gas (and/or CO₂) through
3 orifices spaced along an annular ring (or torus) or at different heights on a ring of vertical pipes.
4 Computer-controlled feedback from the monitoring of gas concentration regulates the feed rate
5 of enriched air to the dispersion pipes. Feedback of wind speed and direction information
6 ensures that the discharges only occur upwind of the treatment plots, and that discharge is
7 restricted or closed down during periods of low wind speed or calm conditions. The diameter of
8 the arrays and their heights (25 to 30 m) in some FACE systems requires large throughputs of
9 enriched air per plot, particularly in forest tree systems. The cost of the throughputs tends to
10 limit the number of enrichment treatments, although Hendrey et al. (1999) argued that the cost
11 on an enriched volume basis is comparable to that of chamber systems.

12 An alternative to the FACE system to free-air fumigation uses a horizontal grid system
13 through which pollutant-enriched air is discharged over the canopies of plants in field plots. The
14 original design, termed the Zonal Air Pollution System (ZAPS), was developed for studying the
15 effects of SO₂ on native grasslands (Lee et al., 1975), and it was later modified by Runeckles
16 et al. (1990) by randomly dividing each of three treatment plots into four subplots, each with
17 different numbers of discharge orifices to provide various levels of O₃ enrichment. With the
18 ZAPS system, changes in wind direction and speed result in varying degrees of carryover from
19 subplot to subplot, effectively resulting in 12 stochastically different seasonal exposures. The
20 system was used for studies of growth effects on field crops and 2- to 4-year old Douglas fir
21 (*Pseudotsuga menziesii*) saplings (Runeckles and Wright, 1996). A larger ZAPS design was
22 used by Wilbourn et al. (1995) on a grass (*Lolium perenne*)-clover (*Trifolium repens*) mixture
23 and by Ollerenshaw et al. (1999) on oilseed rape (*Brassica napus*), whereby four replicate field
24 plots were exposed to intermittent constant additions of O₃ to ambient air. A ZAPS design with
25 eight spatially separated treatment plots was also developed to obtain crop response data used in
26 assessing crop losses in the Fraser Valley, British Columbia, Canada (Runeckles and Bowen,
27 2000).

28 Another recent adaptation of the FACE design was constructed to fumigate soybean
29 with CO₂ and O₃ in combination (Morgan et al., 2004; Rogers et al., 2004). This modified
30 FACE design was based on those of Miglietta et al. (2001) and does not force air through the
31 canopy, instead it relies on wind to disperse air across the fumigation plot.

1 The FACE-type facility developed for the Kranzberg Ozone Fumigation Experiment
2 (KROFEX) in Germany begun in 2000 (Werner and Fabian, 2002) (Nunn et al., 2002) to study
3 the effects of O₃ on mature stands of beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees is
4 more truly a zonal system that functions independently of wind direction. The enrichment of a
5 large volume of the ambient air immediately above the canopy takes place via orifices in vertical
6 tubes suspended from a horizontal grid supported above the canopy.

7 Recognizing the difficulties of modifying the aerial environments of large trees, Tjoelker
8 et al. (1994) devised a free-air system for exposing branches of sugar maple (*Acer saccharum*)
9 trees to O₃. Near the ends of up to 10 branches, enriched air was discharged through small holes
10 in 38-cm-diameter loops of 0.635-cm-OD (outer diameter) teflon tubes positioned 20 to 30 cm
11 below the terminal foliage cluster.

12 Although plume systems make virtually none of the modifications to the physical
13 environment that are inevitable with chambers, their successful use depends on selecting the
14 appropriate numbers, sizes, and orientations of the discharge orifices to avoid hot-spots resulting
15 from the direct impingement of jets of pollutant-enriched air on plant foliage (Werner and
16 Fabian, 2002). However, because mixing is unassisted and completely dependent on wind
17 turbulence and diffusion, local gradients are inevitable even in large-scale FACE systems. Both
18 FACE and ZAPS systems have provisions for shutting down under low wind speed or calm
19 conditions and for an experimental area that is usually defined within a generous border in order
20 to strive for homogeneity of the exposure concentrations within the treatment area. They are
21 also both dependent upon continuous computer-controlled feedback of the O₃ concentrations in
22 the mixed treated air and of the meteorological conditions.

23 24 **AX9.2.2.4 Comparative Studies**

25 All experimental approaches to the exposure of plants to O₃ have shortcomings. The use of
26 laboratory, greenhouse, or field chambers raises concerns for the roles of chamber effects on
27 micrometeorology, as well as the constant turbulence over and within the plant canopy during
28 chamber operation, in modifying O₃ uptake and subsequent plant response. In contrast, plume
29 systems suffer from relatively poor control of exposure levels and an inability to reduce O₃
30 levels below ambient in areas where O₃ is phytotoxic.

1 Although chamber effects vary, one concern is the rise in temperature associated with
2 enclosing plants in a chamber. Still, it is not clear whether these effects are directly related to
3 temperature or are the result of temperature interactions with other environmental variables.
4 For example, Olszyk et al. (1992) undertook a 3-year study of the impact of O₃ on Valencia
5 orange trees (*Citrus sinensis* (L.) Osbeck) in large OTCs to determine if “insidious differences in
6 microclimatic conditions could alter plant growth responses and susceptibility to pollutant
7 stress.” Nonfiltered chambers were found to have somewhat lower average O₃ concentrations
8 than the ambient air, and fewer hourly exceedances of 100 ppb. In cool seasons, stomatal
9 conductance was also lower, implying lower O₃ uptake. However, the cumulative fruit yields
10 were doubled in the chamber trees even though photosynthetically active radiation was
11 consistently reduced by about 19% while leaf temperatures averaged more than 2 °C higher.
12 These data may be somewhat extreme, but they emphasize the need to be cautious when
13 interpreting OTC yield response data, particularly since, as in this study, no O₃ enrichment was
14 involved as a complicating factor.

15 While it is clear that chambers can alter some aspects of plant growth, the question to be
16 answered is whether or not these differences affect plant response to O₃. As noted in the
17 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996), evidence from the comparative
18 studies of OTCs and from closed chamber and O₃-exclusion exposure systems on the growth of
19 alfalfa (*Medicago sativa*) by Olszyk et al. (1986a) suggested that, since significant differences
20 were found for fewer than 10% of the growth parameters measured, the responses were, in
21 general, essentially the same regardless of exposure system used and chamber effects did not
22 significantly affect response. In 1988, Heagle et al. (1988) concluded: “Although chamber
23 effects on yield are common, there are no results showing that this will result in a changed yield
24 response to O₃.” A more recent study of chamber effects examined the responses of tolerant and
25 sensitive white clover clones (*Trifolium repens*) to ambient O₃ in greenhouse, open-top, and
26 ambient plots (Heagle et al., 1996). For individual harvests, greenhouse O₃ exposure reduced the
27 forage weight of the sensitive clone 7 to 23% more than in OTCs. However, the response in
28 OTCs was the same as in ambient plots. Several studies have shown very similar yield response
29 to O₃ for plants grown in pots or in the ground, suggesting that even such a significant change in
30 environment does not alter the proportional response to O₃, at least as long as the plants are well
31 watered (Heagle, 1979; Heagle et al., 1983).

1 Recent evidence obtained using free-air exposure systems and OTCs supports results
2 observed previously in OTC studies (Table AX9-16, Figure AX9-1). Specifically, a series of
3 studies undertaken using free-air O₃ enrichment in Rhinelander, WI (Isebrands et al., 2000,
4 2001) showed that O₃-symptom expression was generally similar in OTCs, FACE, and
5 ambient-O₃ gradient sites, supporting the previously observed variation among trembling aspen
6 clones (*Populus tremuloides* L.) using OTCs (Karnosky et al., 1999). The FACE study
7 evaluated the effects of 3 years of exposure to combinations of elevated CO₂ and O₃ on growth
8 responses in mixture of five trembling aspen clones (Isebrands et al., 2000, 2001). Height,
9 diameter, and stem volume (diameter² × height) were decreased by elevated O₃. On average for
10 all clones, stem volume was decreased by 20% over the 3 years in the elevated O₃ treatment as
11 compared with the 1×-ambient treatment. This FACE facility study is important, because it
12 confirms responses reported previously with the same clones grown in pots or soil in OTCs
13 without the alterations of microclimate induced by chambers. Currently, this is the only U.S.
14 study using this technology to have examined the effects of O₃ under these conditions. This
15 study is also significant, because the elevated O₃-exposure pattern used was intended to
16 reproduce the 6-year average pattern from Washtenaw County, Michigan (Karnosky et al.,
17 1999).

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

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

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

11 **AX9.1.2.5 Ozone Generation Systems**

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

19 **AX9.1.2.6 Experimental Exposure Protocols**

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

25 Hogsett et al. (1985), Lefohn et al. (1986), and others have described the use in controlled
26 chambers of daily exposure profiles based on observed ambient O₃ profiles. Such profiles were
27 used in the elaborately controlled chamber studies of Langebartels et al. (1997), while several
28 recent chamber studies have used simpler computer-controlled half- or full-cosine wave profiles
29 to simulate the typical daily rise and fall in ambient O₃ levels (Mazarura, 1997; McKee et al.,
30 1997a,b).

1 The early studies with OTCs involved adding constant levels of O₃ to ambient air O₃
2 concentrations, but all recent studies have used enrichment delivery systems that maintain
3 proportionality to, and track, ambient O₃ concentrations to produce levels that more closely
4 resemble field observations. Both FACE and ZAPS studies have used proportional enrichment
5 to provide a range of treatments, although Wilbourn et al. (1995) and Ollerenshaw et al. (1999)
6 adjusted their systems manually to obtain a relatively constant target concentration during
7 exposure episodes.

9 **AX9.1.3 Methods Involving Exposures to Ozone in Ambient Air**

10 The experimental methods discussed above are largely aimed at developing quantitative
11 growth-response functions to permit the estimation of the effects of different ambient O₃
12 scenarios. Because such methodologies usually involve exposures to higher than ambient O₃
13 levels, the applicability of the functions obtained may, to some extent, be relevant only to
14 locations that are naturally subjected to high ambient O₃ levels. Furthermore, as pointed out in
15 Section AX9.4, the response functions that they generate rarely incorporate other environmental,
16 genetic, and physiological factors, many of which can severely modify the magnitude of the
17 response to O₃. The consequences of ignoring such modifications have been well stated by
18 De Santis (1999). The European level for protecting crops (based on the AOT40 index; see
19 Section AX9.4) was derived from OTC studies of O₃-induced yield loss in wheat observed in
20 experiments conducted mostly in non-Mediterranean locations. However, the impact of
21 ambient O₃ on wheat yields in the Po Valley of northern Italy is much less than the devastatingly
22 high loss (>60%) suggested by the seasonal exceedances of the level. On a similar note,
23 Manning (2003) has recently urged the absolute necessity of seeking “ground truth” as
24 verification of the nature and magnitude of impacts on vegetation as suggested by response
25 functions using ambient O₃ monitoring data.

26 Such concerns clearly show that attention needs to be focused on incorporating
27 consideration of environmental and other factors into the response functions upon which
28 standards are based. This will require the development of improved simulation response models.
29 These concerns have also led to increasing attention being paid to seeking and developing
30 alternative approaches to assessing of impact, and the geographic extent of such impact —
31 approaches that are based on in situ exposures to ambient or sub-ambient O₃ levels. Although

1 one approach, the use of air-exclusion systems, requires experimental facilities, the other
2 approaches are generally based on simple field observations or measurements and, hence, can be
3 undertaken on a wide geographic scale.

4 5 **AX9.1.3.1 Air-Exclusion Systems**

6 The term, air-exclusion system, usually refers to a chamberless field system specifically
7 designed to protect plants from exposure to polluted air by blowing filtered air through their
8 canopies. Hogsett et al. (1987a,b) described several dedicated systems developed in the 1960s
9 and 1970s, but there appear to have been no recent O₃-exclusion studies using systems
10 specifically designed for this purpose since those described by Olszyk et al. (1986a,b). Their
11 system, a modification of the earlier system of Jones et al. (1977), consisted of perforated 31.8-
12 cm OD inflatable polyethylene tubes laid between crop rows and supplied with CF air.
13 By increasing the size of the orifices progressively in sections along the 9-m length of the tubes,
14 an exclusion gradient was created with a progressive decrease in O₃ levels in the air surrounding
15 the crop from one end of the system to the other. The system was used for studies on alfalfa
16 comparing plant response in OTCs, closed field chambers, the air-exclusion system, and ambient
17 air plots (as discussed above in Section AX9.1.2.4).

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

21 22 **AX9.1.3.2 Natural Gradients**

23 Naturally occurring O₃ gradients hold potential for the examination of plant responses
24 along the gradient. However, few such gradients can be found which meet the rigorous
25 statistical requirements for comparable site characteristics such as soil type, temperature,
26 rainfall, radiation, and aspect (Manning and Krupa, 1992); although with small plants, soil
27 variability can be avoided by the use of potted plants. The use of soil monoliths transported to
28 various locations along natural O₃ gradients is another possible approach to overcome
29 differences in soils; however, again this approach is limited to small plants.

30 Studies in the 1970s used the natural gradients occurring in southern California to assess
31 yield losses of alfalfa and tomato (*Lycopersicon esculentum* L.) (Oshima et al., 1976, 1977).

1 A transect study of the impact of O₃ on the growth of white clover and barley (*Hordeum vulgare*
2 L.) in the United Kingdom was confounded by differences in the concurrent gradients of SO₂
3 and NO₂ pollution (Ashmore et al., 1988). Studies of forest tree species in national parks in the
4 eastern United States (Winner et al., 1989) revealed increasing gradients of O₃ and visible foliar
5 injury with increased elevation.

7 **AX9.1.3.3 Use of Chemical Protectants**

8 The use of protective chemicals is a relatively inexpensive, promising alternative to
9 experimental field exposures in chambers or free-air systems for determining plant response
10 to O₃. These chemicals have recently been used in studies of different plant species, both in the
11 United States (Bergweiler and Manning, 1999; Kuehler and Flagler, 1999) and in Europe
12 (Bortier et al., 2001a; Pleijel et al., 1999; Wu and Tiedemann, 2002), to determine if ambient O₃
13 concentrations affect plant growth and productivity or are just exacerbating foliar injury.
14 Several chemical compounds (e.g., antioxidants, antisenescence agents, fungicides, pesticides)
15 have been known for many years to provide plants some protection from photochemical oxidants
16 such as O₃ (Manning and Krupa, 1992). Most of these chemicals were originally used as a one-
17 time application to reduce visible injury caused by acute O₃ exposures. The most widely used
18 and popular of these has been ethylenediurea (EDU). Carnahan et al. (1978) reported that EDU
19 protected pinto bean (*Phaseolus vulgaris*) from acute O₃ injury. After this initial investigation,
20 EDU was shown to suppress visible O₃ injury on several species of plants under both controlled
21 and field conditions (Brennan et al., 1987; Clarke et al., 1983). However, due to lack of a
22 commercial market for this product, its commercial manufacture was largely discontinued.
23 Other chemicals, including benomyl (Manning et al., 1974), carboxin (Rich et al., 1974),
24 ascorbic acid (Dass and Weaver, 1968), and others (Manning and Krupa, 1992), also exhibited
25 some beneficial effects in reducing visible O₃ injury.

26 Several recent studies have used EDU in assessing the response of several plant species
27 to O₃ to help validate the proposed critical level (AOT40 = 3000 ppb.h; see Section AX9.5) for
28 crop protection in Europe (Ball et al., 1998; Ribas and Penuelas, 2000; Tonneijck and Van Dijk,
29 2002a,b). EDU appeared to provide protection from visible foliar injury, but the results
30 regarding yield and biomass reductions were mixed. In a 3-year study over 12 sites throughout
31 Europe, Ball et al. (1998) used the ratio of EDU-treated versus non-treated white clover biomass

1 and did not find a significant relationship between biomass reductions and AOT40 level.
2 However, an artificial neural network (ANN) model including vapor pressure deficit (VPD),
3 temperature, longitude, year, and altitude explained much more of the variance ($r^2 = 0.79$). The
4 authors suggested that the greater sensitivity at certain sites in Germany may have been due to
5 occurrence of other pollutants. This meta-analysis indicates that EDU effects may be influenced
6 substantially by environmental factors.

7 In another study, Tonneijck and Van Dijk (2002b) assessed the relationship of visible
8 injury of subterranean clover (*Trifolium subterraneum*) to ambient O₃ at four sites over three
9 growing seasons in the Netherlands, using EDU-treated and nontreated plants. Visible injury
10 varied by site and year, but was reduced to near zero by EDU treatment. However, no
11 relationship indicative of a protective effect of EDU with this plant species was observed for
12 biomass. Tonneijck and Van Dijk (2002a) also reported similar results with pinto bean. Both
13 EDU-treated and nontreated plants were exposed to ambient O₃ at three locations in Spain over
14 one growing season (Ribas and Penuelas, 2000). Reductions in yield and biomass were
15 correlated with O₃ concentration and EDU provided some protective effect, although results
16 varied by location and with meteorological conditions.

17 Chemicals have also been used to assess the effects of O₃ on tree species. Bortier et al.
18 (2001b) injected seedlings of an O₃-sensitive poplar (*Populus nigra*) clone with EDU and
19 measured growth over a 1-year period at a field site near Brussels, Belgium. Over the growing
20 season, stem diameter increment was significantly higher (16%), biomass was increased (9%),
21 and foliar O₃ symptoms were slightly less for the EDU-treated seedlings. Ozone levels were
22 reported to be low (AOT40 = 6170 ppb.h, May to September) during the exposure period.
23 In another study, Manning et al. (2003) applied EDU (foliar spray) and sodium erythorbate
24 (NaE) at various concentrations, biweekly for three growing seasons to loblolly pine (*Pinus*
25 *taeda*) at a field site in east Texas. After 3 years, the trees were harvested and biomass
26 measured. Neither EDU nor NaE prevented foliar O₃ injury, but EDU applications at 450 ppm
27 resulted in increases in stem diameter and height, and total above-ground biomass. These
28 measures of growth also tended to slightly increase with applications of NaE, but the effects
29 were statistically nonsignificant.

30 The mechanisms by which protective chemicals, especially EDU, protect plants are poorly
31 understood. However, Wu and von Tiedemann (2002) reported that applications of two recently

1 developed fungicides (azoxystrobin and epoxiconazole) provided protection to spring barley to
2 relatively high O₃ exposures (150 to 250 ppb, 5 days, 7h/day) and resulted in increases in leaf
3 soluble protein content as well as the activity of several antioxidative enzymes (e.g., superoxide
4 dismutase, catalase, ascorbate-peroxidase, and glutathione reductase). In addition, the increase
5 in these enzymes reduced superoxide levels in the leaves.

6 Despite advances in the use of protective chemicals, a number of hurdles remain in using
7 them for assessing O₃ effects. The phytotoxicity of EDU is well known, and the point has been
8 made repeatedly that for a particular species or cultivar, tests under a range of environmental
9 conditions and O₃ exposures must be made to establish the efficacy of EDU for quantifying O₃
10 effects (Heggestad, 1988; Kostka-Rick and Manning, 1992a). Unfortunately, although many
11 studies with EDU have been conducted in recent decades, very few have used multiple EDU
12 application levels along with multiple O₃ exposures to characterize the EDU system for a given
13 plant species.

14 Recent studies have also shown that EDU does not always have greater effects at higher O₃
15 exposures. For pinto bean grown in pots in studies in Spain and in the Netherlands, EDU
16 increased pod yield (Ribas and Penuelas, 2000; Tonneijck and Van Dijk, 1997). However, this
17 effect was not greater at sites with higher O₃ exposure despite consistent experimental protocols
18 at all sites, including growing the same cultivar in pots with adequate water (Ribas and Penuelas,
19 2000; Tonneijck and Van Dijk, 1997). Such results suggest that it may be difficult to quantify
20 ambient O₃ effects using EDU, because the amount of plant growth or yield expected at a low
21 (background) O₃ concentration cannot be inferred from EDU-treated plants grown at locations
22 with higher O₃ exposures.

23 Several studies suggest that EDU has effects other than its antioxidant protection and
24 phytotoxicity and show that environmental conditions affect the degree of protection afforded by
25 protective chemicals. In one study, even low concentrations of EDU (8 to 32 mg L⁻¹ soil),
26 decreased soybean yield under low O₃ exposure (7-h mean of 19 ppb) in CF OTCs (Miller et al.,
27 1994). This study also demonstrated that phytotoxicity (both foliar symptoms and growth
28 effects) can differ even in the same series of experiments, apparently due to changes in
29 environmental conditions, and that EDU can suppress yield at application rates that do not
30 always cause foliar symptoms (Miller et al., 1994). Finally, this study found that EDU altered
31 biomass partitioning by increasing vegetative growth and decreasing reproductive growth.

1 A study of pinto bean grown in OTCs in Germany found that EDU treatment in CF OTCs
2 significantly increased yield, while EDU had no significant effect on yield in other O₃ treatments
3 (Brunschon-Harti et al., 1995). In this study, O₃ significantly reduced the mass of pods, shoots,
4 and roots. EDU increased root, leaf, and shoot mass across O₃ treatments. However, the only
5 statistically significant interaction occurred with O₃ × root mass. This study indicates that EDU
6 can stimulate above-ground growth and/or delay senescence regardless of O₃ treatment.

7 The EDU approach for assessing the impact of ambient O₃ exposures is potentially useful,
8 because it provides a separate line of evidence from other methods. Before using these
9 chemicals in a field setting, preliminary investigations under controlled conditions (e.g.,
10 chambers) should be done to evaluate the methods and timing of application, as well as proper
11 application rates, so as to avoid any potential toxic effects (Manning, 2000; Manning and Krupa,
12 1992). Unfortunately, such characterization has so far been limited, although substantial
13 progress has been made for radish (*Raphanus sativus* L.) (Kostka-Rick et al., 1993; Kostka-Rick
14 and Manning, 1992a, 1993). Thus, it is difficult to use data from existing EDU studies to
15 develop exposure-response relationships or to quantify the effects of ambient O₃ exposure.
16 Despite these limitations, the EDU studies reviewed in previous O₃ AQCDs (U.S. Environmental
17 Protection Agency, 1986, 1996) and the more recent studies summarized in Table AX9-1
18 (Section AX9.5) provide another line of evidence that ambient O₃ exposures occurring in many
19 regions of the United States may be reducing the growth of crops and trees.

21 **AX9.1.3.4 Biomonitoring**

22 ***Bioindicators***

23 The use of biological indicators to detect the presence of O₃ injury to plants is a
24 longstanding and effective methodology (Chappelka and Samuelson, 1998; Manning and Krupa,
25 1992). A bioindicator can be defined as a vascular or nonvascular plant exhibiting a typical and
26 verifiable response when exposed to a plant stress such as an air pollutant (Manning et al., 2003).
27 To be considered a good indicator species, plants must

- 28 (1) exhibit a distinct, verified response,
- 29 (2) have few or no confounding disease or pest problems, and
- 30 (3) exhibit genetic stability.

Table AX9-1. Advantages and Disadvantages of Protective Chemicals Used in Assessment of O₃ Effects on Plants

Advantages
No chambers required. Plants exposed to ambient conditions of O ₃ , light, temperature, etc.
Can conduct studies “in situ.” Equipment needs are minimal. No “chamber effects”
A high degree of replication possible both within and among locations

Disadvantages
Exposure-response studies require inclusion of other methodologies (OTCs, etc.)
Need measurements of ambient O ₃ and other meteorological variables (temp, rainfall, etc)
Many are toxic; have to conduct preliminary toxicology studies to determine proper rate, timing etc.
Species response can vary; need to screen for proper species to use
Mode of action not fully understood; may alter growth and biomass partitioning

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

1 Such sensitive plants can be used to detect the presence of a specific air pollutant such
2 as O₃ in the ambient air at a specific location or region and, as a result of the magnitude of their
3 response, provide unique information regarding specific ambient air quality. Bioindicators can
4 be either introduced *sentinels*, such as the widely used tobacco (*Nicotiana tabacum*) variety
5 Bel W3, or *detectors*, which are sensitive native plant species (e.g., milkweed [*Asclepias*
6 *syriaca*]). The approach is especially useful in areas where O₃ monitors are not operated
7 (Manning et al., 2003). For example, in remote wilderness areas where instrument monitoring is
8 generally not available, the use of bioindicator surveys in conjunction with the use of passive
9 samplers (Krupa et al., 2001) is a particularly useful methodology (Manning et al., 2003).
10 However, the method requires expertise or training in recognizing those signs and symptoms
11 uniquely attributable to exposure to O₃ as well as in their quantitative assessment.

12 Since the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) many new
13 sensitive species have been identified from controlled exposure studies and verified in the field
14 (Flagler, 1998; Innes et al., 2001). In addition, several new uses of this methodology have been
15 demonstrated, including a national O₃ bioindicator network, studies in wilderness areas, and

1 mature tree studies. Although it has been difficult to find robust relationships between the foliar
2 injury symptoms caused by O₃ and effects on plant productivity or ecosystem function, visible
3 injury correlations with growth responses have been reported (Table AX9-2) (1998) (2003)
4 (2003). One workshop on the utility of bioindicators of air pollutants led to a useful series of
5 peer-reviewed publications in *Environmental Pollution* (Skelly, 2003).
6
7

**Table AX9-2. Advantages and Disadvantages of Bioindicators Used to Study
O₃ Plant Effects**

Advantages
No chambers required. Plants exposed to ambient conditions of O ₃ , light, temperature, etc.
Relatively inexpensive. Equipment needs are minimal. No “chamber effects”
A high degree of replication possible (sentinels) both within and among locations

Disadvantages
Results are generally correlative in nature with no true control
Individuals need to be trained and experienced in O ₃ symptom recognition
Need adequate numbers of plants (detectors) to ensure valid results
Need preliminary tests to insure a constant symptomatology of material used
Need to use more than one indicator species (detector) per area if possible
Need to quantify site characteristics (soils, light) that may influence symptom expression
Need measurements of ambient O ₃ (active or passive) and other meteorological variables (temp, rainfall, etc)
Need to ensure that cultural (sentinels) practices (soil, irrigation, fertilization, etc.) are similar among sites

1 ***National network***

2 The U.S. Forest Service in cooperation with other federal and state agencies developed a
3 network of O₃ bioindicators to detect the presence of O₃ in forested systems throughout the
4 United States (Smith et al., 2003). This ongoing program was initiated in 1994; and 33 states
5 currently participate. In a coordinated effort, a systematic grid system is used as the basis of plot

1 selection, and field crews are trained to evaluate O₃ symptoms on sensitive plant species within
2 the plots (Coulston et al., 2003; Smith et al., 2003).

3 The network has provided evidence of O₃ concentrations high enough to induce visible
4 symptoms on sensitive vegetation. From repeated observations and measurements made over a
5 number of years, specific patterns of areas experiencing visible O₃ injury symptoms can be
6 identified. Coulston et al. (2003) used information gathered over a 6-year period (1994 to 1999)
7 from the network to identify several species that were sensitive to O₃ over a regional scale
8 including sweetgum (*Liquidambar styraciflua*), loblolly pine, and black cherry (*Prunus*
9 *serotina*).

11 ***Wilderness areas***

12 The use of bioindicator species as detectors has proven to be an effective technique for
13 deriving a relative estimate of O₃ injury in wilderness areas in both the United States and Europe
14 (Chappelka et al., 1997, 2003; Manning et al., 2002). However, to be truly effective, these
15 regional and national bioindicator studies need the inclusion of air quality data and related
16 growth studies to determine effects on productivity and ecosystem function (Bytnerowicz et al.,
17 2002; Manning et al., 2003; Smith et al., 2003). In addition, O₃ often co-occurs with other air
18 borne pollutants, so it is important to consider that, in some areas, other pollutants may be
19 playing a role as well.

20 Chappelka et al. (1997, 2003) conducted surveys of foliar injury on several native plant
21 species throughout the Great Smoky Mountains National Park (GRSM), including black cherry
22 (*Prunus serotina*), tall milkweed (*Asclepias exaltata*), cutleaf coneflower (*Rudbeckia laciniata*),
23 and crownbeard (*Verbesina occidentalis*). Visible foliar symptoms were prevalent throughout
24 the Park, indicating that injury-producing O₃ levels were widespread in GRSM.

25 Manning et al. (2002) recently summarized a multiyear (1993 to 2000) bioindicator project
26 in the Carpathian Mountain range in eastern Europe. They evaluated numerous trees, shrubs,
27 forbs, and vines for possible symptoms of O₃ injury. Observations were made at plots located in
28 the vicinity of either active or passive O₃ monitors (Bytnerowicz et al., 2002). Approximately
29 30 species of native plant detectors were identified as possible bioindicators, the majority of
30 which (21) were shrubs (Manning et al., 2002). Based on these observations, it was concluded
31 that O₃ concentrations were sufficiently high to impact ecosystems in the region. Similar

1 investigations regarding the sensitivity of native species have been conducted in Switzerland
2 (Novak et al., 2003) and Spain (Orendovici et al., 2003).

4 ***Mature tree detectors***

5 Many studies have reported visible injury of mature coniferous trees caused by O₃,
6 primarily in the western United States (Arbaugh et al., 1998) and, to a lesser extent, to mature
7 deciduous trees in eastern North America. In an effort to determine the extent and magnitude of
8 visible injury in mature tree canopies, Hildebrand et al. (1996) and Chappelka et al. (1999b)
9 conducted independent studies in the GRSM and the Shenandoah National Park (SHEN).
10 The species examined were sassafras (*Sassafras albidum*), black cherry, and yellow-poplar
11 (*Liriodendron tulipifera* L.) in GRSM and white ash (*Fraxinus americana* L.), black cherry, and
12 yellow-poplar in SHEN. Protocols were similar at both parks, and trees were located near O₃
13 monitors at three different areas in each park. Results from both studies indicated that symptoms
14 of O₃ injury were present in the trees and correlated with O₃ exposure both spatially and
15 temporally. Ozone injury tended to be most severe at the highest elevation, except with yellow-
16 poplar.

17 Hildebrand et al. (1996) observed significant O₃ exposure-plant response relationships with
18 black cherry. The best relationships were found between foliar injury and the cumulative
19 exposure statistics SUM06 and W126 (see Section AX9.5), indicating that higher O₃
20 concentrations were important in eliciting a response in black cherry. No O₃ exposure-plant
21 response relationships were found with any species tested in GRSM (Chappelka et al., 1999b);
22 but, when the data were combined for both parks, a significant correlation ($r = 0.72$) with black
23 cherry was found for both SUM06 and W126, and injury was the greatest ($r = 0.87$) at the higher
24 elevations (Chappelka et al., 1999a).

25 Based on a study in which visible symptoms of O₃ injury were characterized for large,
26 mature yellow-poplar and black cherry trees in GRSM (Chappelka et al., 1999a), Somers et al.
27 (1998) compared radial growth differences among trees classified as sensitive or nonsensitive
28 based on the severity of visible foliar injury observed over a 3-year period (1991 to 1993).
29 Significantly more radial growth was observed over both a 5- and a 10-year period for the
30 nonsensitive compared to the sensitive trees. No significant relationship was found for black
31 cherry tree growth.

1 Vollenweider et al. (2003), using data collected from continuous forest inventory (CFI)
2 plots across Massachusetts, compared growth rates among either symptomatic or asymptomatic
3 mature black cherry trees. Of the 120 trees sampled in 1996, 47% exhibited visible foliar injury.
4 Using CFI data, growth rates were compared over a 31-year period. The growth rates for
5 symptomatic trees were reduced by 28% compared with asymptomatic trees.

6 Because these studies (Somers et al., 1998; Vollenweider et al., 2003) were not controlled
7 studies and used a small sample of trees, they cannot validly be used to characterize cause and
8 effects related to the visible symptoms and radial growth they describe. However, the results
9 indicate the *possibility* that O₃ is correlated with growth losses in some sensitive genotypes,
10 illustrating the potential usefulness of this visible O₃ injury methodology in assessing effects on
11 the growth rates of mature deciduous trees.

13 ***Cultivar comparisons***

14 The idea of using cultivars or isogenic lines of crop species that differed in O₃ sensitivity as
15 sentinels to determine the ambient effects of O₃ in the field was presented in the 1996 O₃ AQCD
16 (U.S. Environmental Protection Agency, 1996). The rationale was that comparing the ratio of
17 injury scores or some measure of growth between two different cultivars varying in O₃
18 sensitivity should be indicative of the relative amount of ambient stress to plants at a given
19 location. A sensitive:resistant ratio close to unity would indicate relatively low O₃
20 concentrations; and a low ratio, higher O₃ levels. Results from locations differing in O₃
21 concentrations could be evaluated to develop exposure-response models. The original protocol
22 was derived using two isogenic lines of white clover (*Trifolium repens*) differing in O₃
23 sensitivity (Heagle et al., 1994b, 1995).

24 This white clover model system has been used in several multi-location studies in the
25 United States (Heagle and Stefanski, 2000) and Europe (Ball et al., 2000; Bermejo et al., 2002;
26 Mills et al., 2000). Heagle and Stefanski (2000) compared results from eight sites over a 2-year
27 period with various exposure indices (SUM00, SUM06, W126, and others) to determine a
28 best-fit regression. They found that most of the indices performed similarly. The highest r²
29 values (0.87 to 0.93) were obtained using only the later harvests and a 6 h day⁻¹ index (1000 to
30 1600 h). Similar multiple-comparison studies conducted in Europe using the AOT40 index (Ball
31 et al., 2000; Mills et al., 2000) yielded poorer r² values. Factors such as air temperature, NO_x

1 (high levels at some sites), and lower O₃ concentrations in Europe were suggested to account in
2 part for the differences between U.S. and European study results. Bermejo et al. (2002), in a
3 study in Spain, improved the model by comparing the biomass ratio of these white clover
4 isolines to measures of O₃ uptake (flux) rather than an exposure index (AOT40). Together, these
5 studies indicate that systems such as the white clover model can help reveal O₃ exposure-
6 response relationships and provide valuable information regarding ambient O₃ conditions in a
7 given location. Table AX9-3 lists the advantages and disadvantages of the use of cultivar
8 comparisons in assessing O₃ effects of plants.
9
10

Table AX9-3. Advantages and Disadvantages of Cultivar Comparisons Used in Assessment of O₃ Effects on Plants

Advantages

No chambers required. Plants exposed to ambient conditions of O₃, light, temperature, etc.
Relatively inexpensive. Equipment needs are minimal. No “chamber effects”
A high degree of replication possible both within and among locations
Can conduct studies “in situ”

Disadvantages

Need preliminary tests to insure sensitivity and growth patterns of genotypes used are consistent
Need measurements of ambient O₃ and other meteorological variables (temp, rainfall, etc)
Have to ensure cultural practices (soil, irrigation, fertilization, etc.) are similar among sites
Need to closely monitor plants for presence of other factors that may cause a misinterpretation of results

Dendrochronological techniques

1 It has been difficult to determine whether O₃ significantly affects tree growth and
2 productivity in the field, because O₃ concentrations are omnipresent and tree response to this
3 pollutant is altered by many factors. The use of dendrochronological techniques to answer
4 questions regarding ambient O₃ effects on forest growth and ecosystem function has recently
5 emerged as a very useful biomonitoring methodology (Cook, 1990; McLaughlin et al., 2002).
6

1 The technique is useful when either instrument or passive O₃ monitoring methods are used to
2 determine ambient O₃ conditions.

3 Initial experiments were primarily correlative in nature and attempted to relate symptoms
4 of visible injury with growth losses as revealed by tree ring analysis (Arbaugh et al., 1998;
5 Benoit et al., 1983; Peterson et al., 1995; Somers et al., 1998; Swank and Vose, 1990). These
6 studies evaluated radial growth patterns determined by cores removed from trees in the presence
7 or absence of overt O₃ injury symptoms.

8 The method has also been adapted to better understand forest ecosystem function
9 (McLaughlin and Downing, 1995; Bartholomay et al., 1997; McLaughlin et al., 2003). The
10 response of mature loblolly pine growing in eastern Tennessee to ambient O₃ and moisture stress
11 was evaluated by McLaughlin and Downing (1995, 1996). They made radial growth
12 measurements from 12 to 37 times per year using dendrometer bands and determined
13 relationships between O₃, moisture stress, and radial growth. Exposures to O₃ concentrations
14 ≥0.04 ppm with high temperatures and low soil moisture resulted in short-term depression in
15 radial growth. Reductions in growth were estimated to vary from 0 to 15% per year and
16 averaged approximately 5% per year.

17 Bartholomay et al. (1997) examined white pine (*Pinus strobus*) radial growth in eight
18 stands throughout Acadia National Park, Maine over a 10-year period from 1983 to 1992. They
19 related growth rates to several factors, including O₃ concentration. Ozone levels were negatively
20 correlated with radial growth in seven of the eight stands. Site characteristics were important in
21 the relationship: stands growing on shallow, poorly drained soils were most sensitive to O₃ in
22 the late portion of the growing season, possibly due to premature senescence of foliage.
23 However, litterfall measurements were not reported. Trees growing on better sites were more
24 sensitive to O₃ during the entire growing season, indicating the possibility of high O₃ uptake
25 rates throughout the growing season. Although these field studies (Bartholomay et al., 1997,
26 1996; McLaughlin and Downing, 1995) did not compare the direct effects of O₃ on the two pine
27 species, they indicate that potential interactions exist among O₃ and other climatic and edaphic
28 factors, such as temperature and soil moisture.

29 Using both automated and manual dendrometer bands, McLaughlin et al. (2002) examined
30 the growth response of yellow-poplar trees recently released from competition. In addition to
31 measuring growth, sap flow measurements were conducted and soil moisture was measured in

1 the vicinity of the trees. They were not able to detect O₃ effects in this 1-year study. Advantages
2 and disadvantages of dendrochronology techniques for evaluating whole-tree physiological
3 responses for individual trees and forest stands are listed in Table AX9-4.
4
5

**Table AX9-4. Advantages and Disadvantages of Various Dendrochronological
Techniques Used in Assessment of O₃ Effects on Plants**

Advantages
Provide information regarding growth effects under ambient conditions
Good historical information regarding O ₃ effects
Can provide data on daily and seasonal growth and O ₃ patterns and correlate with physiological function
Provide information on forest function related to ambient O ₃ concentrations
Can link data with process-level growth models

Disadvantages
Results are generally correlative in nature with no true control
Need background O ₃ and meteorological data (historical records)
Need to account for other factors such as competition, in analyzing data
Individuals need to be trained in counting growth rings
Replication can be difficult (expensive and technological limitations)
Complicated statistical analyses are sometimes required
Can be expensive, especially if using automated growth (dendrometer) bands

1 The use and evolution of various dendrochronological methods in the field of air pollution
2 effects research is reviewed in detail by McLaughlin et al. (2002). Automated dendrometer
3 bands provide a powerful tool for measuring radial growth responses of trees on an hourly or
4 daily basis. Diurnal patterns of growth can be related to water use and O₃ concentrations using
5 time-series analyses. The major drawbacks of the method are that it is expensive and time
6 consuming.
7

1 **AX9.1.3.5 Calibrated Passive Monitors**

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

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

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

27 28 **AX9.1.4 Numerical/Statistical Methodologies**

29 Proper experimental design strategies including replication, randomization, and
30 experimental protocols are paramount in O₃-effects research. These have been discussed in
31 detail in previous O₃ AQCDs (U.S. Environmental Protection Agency, 1996, 1986), as have the

1 different statistical analytical procedures used to determine the probable significance of results.
2 However, new investigative approaches have demanded the adoption of new analytical methods.
3 For example, the use of dendrochronological techniques has led to the use of time-series analysis
4 (McLaughlin et al., 2003) and linear aggregate models (Cook [1990], as reviewed by
5 McLaughlin et al. [2002]).

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

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

20 The meta-analysis method clearly has the potential to consolidate and refine the
21 quantitative exposure-response models for many species. The majority of the reported growth
22 and physiological responses related to O₃ stress are for individual plants, primarily in various
23 types of exposure chambers. It is difficult to extrapolate these responses to stand/community,
24 ecosystem, or region-wide assessments, particularly in view of the importance of the significant
25 interactions that may occur between plant responses O₃ and other environmental stresses. Along
26 with the shift in effects research to a more ecological approach, these concerns necessitate a
27 move from simple regression analysis to more complex mathematical approaches to handle a
28 wider array of independent input variables than O₃ exposure alone. Other independent input
29 variables that must be accounted for include air and soil temperatures, soil moisture, relative
30 humidity, wind speed, and, particularly in the case of natural systems, biotic factors such as pests
31 and pathogens, plant density/spacing, and measures of plant competition.

1 Artificial neural network methodology was used by Balls et al. (1995) for “unraveling the
2 complex interactions between microclimate, ozone dose, and ozone injury in clover” and in the
3 study with the protectant chemical EDU, discussed in Section AX9.2.3.3 (Ball et al., 1998). The
4 multi-factor model for predicting the effects of ambient O₃ on white clover developed by Mills
5 et al. (2000) utilized both ANN and multiple linear regression methods.

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

21
22
**Table AX9-5. Advantages and Disadvantages of Modeling Techniques Used in
Assessment of O₃ Effects on Plants**

Advantages

- Provide an understanding of cause-effect relationships over time
-

Disadvantages

- Have to make assumptions based on a paucity of data
 - Most models are very complex and difficult to understand
 - Need to be evaluated for predictive validity
-
-

1 **AX9.1.5 Improved Methods for Defining Exposure**

2 Ambient air quality is defined in terms of the measured O₃ concentrations in the air at plant
3 height above ground level. Compilations of such concentration data have long been used as
4 surrogates of the exposures to which plants are subjected. However, as long ago as 1965, field
5 research provided evidence that plant response was a function, not of ambient O₃ concentration
6 per se, but of the estimated flux of O₃ to the plant canopy (Mukammal, 1965). Subsequently,
7 Runeckles (1974) introduced the term “effective dose” to define that part of the ambient
8 exposure that was taken up by a plant. Fowler and Cape (1982) later referred it as “pollutant
9 applied dose” (PAD), defined as the product of concentration, time and stomatal (or canopy)
10 conductance, with units g m⁻². Such estimates of O₃ uptake or flux provide a more biologically
11 relevant description of exposure than the simple product of concentration and time alone, and
12 they formed the basis of Reich’s 1983 “unifying theory” of plant response to O₃ (Reich, 1983).

13 However, it was not until the early 1990s that the inherent advantages of using O₃ flux
14 rather than O₃ concentration as a basis for determining response effects began to be widely
15 accepted, as demonstrated by the subsequent increase in publications involving flux
16 measurements and modeling (e.g., Fuhrer et al. [1997]; Grünhage and Jäger [2003]); Grünhage
17 et al. [1993; 1997]; Massman et al. [2000]); Musselman and Massman [1999]; Pleijel [1998]).
18 A key requirement for flux determination is the measurement of stomatal or canopy
19 conductances, using established porometer/cuvette techniques or eddy correlation methods.
20 The usefulness and relevance of flux as a measure of exposure are discussed in detail in Section
21 AX9.4.

22 Efforts to develop regional-scale models of O₃ deposition and stomatal uptake are currently
23 under way with a view to providing improved assessments of the risks to vegetation across
24 Europe (Emberson et al., 2000; Simpson et al., 2001, 2003).
25
26

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1 **AX9.2 SPECIES RESPONSE/MODE-OF-ACTION**

2 **AX9.2.1 Introduction**

3 The evaluation of O₃ risk to vegetation requires fundamental understanding of both the
4 functioning of the vegetation and how external environmental influences can alter that function.
5 For biological organisms subjected to atmospheric O₃, those alterations can be complex and
6 multiple. In addition, biological organisms have plasticity to external interactions due to their
7 complex internal, self-correcting systems, making the task of identifying their “correct”
8 functioning difficult. This section emphasizes reactions of O₃ with the cell and tissue, rather
9 than the whole plant, to describe the fundamental mechanisms known to govern the response of
10 the plant to O₃ exposure.

11 The many regulatory systems contained in leaves change both as a function of leaf
12 development and in response to various environmental stresses. Leaves function as the major
13 regulators of anatomical and morphological development of the shoot and control the
14 translocation of carbohydrates to the whole plant (Dickson and Isebrands, 1991). This section
15 discusses the movement of O₃ into plant leaves and their biochemical and physiological
16 responses to O₃.

17 The 1996 criteria document (U.S. Environmental Protection Agency, 1996) assessed the
18 information available at that time concerning the biochemical and physiological responses to the
19 movement of O₃ into plant leaves. This information continues to be valid. Ozone uptake in a
20 plant canopy is a complex process involving adsorption to surfaces (leaves, stems, and soil) and
21 absorption into leaves (Figure AX9-1). However, the initial biochemical changes that result
22 within leaf cells after the entry of O₃ and how these changes interact to produce plant responses
23 remain unclear. The response of vascular plants to O₃ may be viewed as the culmination of a
24 sequence of physical, biochemical, and physiological events. Only the O₃ that diffuses into a
25 plant through the stomata (which exert some control on O₃ uptake) to the active sites within a
26 leaf impairs plant processes or performance. An effect will occur only if sufficient amounts
27 of O₃ reach sensitive cellular sites that are subject to the various physiological and biochemical
28 controls within the leaf cells. Ozone injury will not occur if (1) the rate and amount of O₃ uptake
29 is small enough for the plant to detoxify or metabolize O₃ or its metabolites or (2) the plant is
30 able to repair or compensate for the O₃ impacts (Tingey and Taylor, 1982; U.S. Environmental
31 Protection Agency, 1996). Therefore, a precondition for O₃ to affect plant

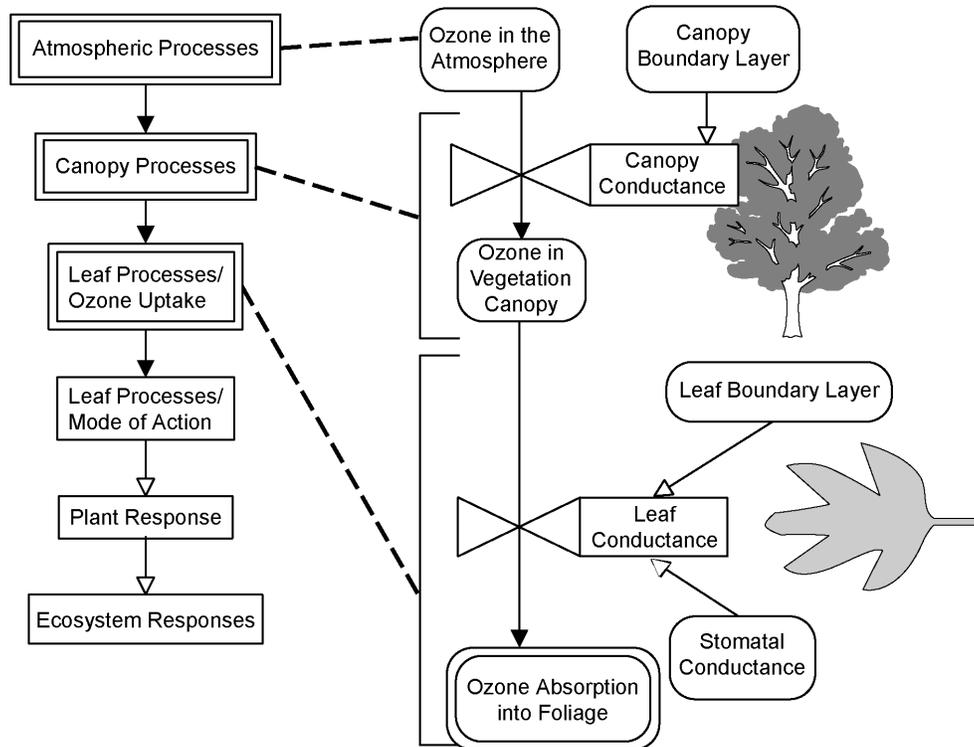


Figure AX9-1. Ozone uptake from the atmosphere. Ozone moves from the atmosphere above the canopy boundary layer into the canopy primarily by turbulent air flow. Canopy conductance, controlled by the complexity of the canopy architecture, is a measure of the ease with which gases move into the canopy. Within the canopy, O_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_3 flow; dotted arrows indicate processes affecting uptake or response to O_3 . Boxes at the left with double borders are those processes described in the figure.

1 function is that it must enter the stomata and be absorbed into the water lining the mesophyll cell
 2 walls. The response of each plant is determined by the amount of O_3 entering the leaves, which
 3 varies from leaf to leaf.

4 Some potentially significant processes have been investigated since the 1996 criteria
 5 document, especially detoxification and compensatory processes. The role of detoxification in
 6 providing a level of resistance to O_3 has been investigated; however, it is still not clear as to what
 7 extent detoxification can protect against O_3 injury. Data are needed especially on the potential

1 rates of antioxidant production and on the subcellular localization of the antioxidants. Potential
2 rates of antioxidant production are needed to assess whether they are sufficient to detoxify the O₃
3 as it enters the cell. The subcellular location(s) is needed to assess whether the antioxidants are
4 in cell wall or plasmalemma locations that permit contact with the O₃ before it has a chance to
5 damage subcellular systems. Although these processes divert resources away from other sinks,
6 detoxification and compensation processes may counteract the reduction in canopy carbon
7 fixation caused by O₃. The quantitative importance of these processes requires investigation.

8 As a result of the research since the 1996 criteria document (U.S. Environmental Protection
9 Agency, 1996), the way in which O₃ exposure reduces photosynthesis, especially its effects on
10 the central carboxylating enzyme, Rubisco (ribulose-1,6-P₂-carboxylase/oxygenase1), is
11 better understood. The rate of leaf senescence has been shown to increase as a function of
12 increasing O₃ exposure. The mechanism of the increased senescence is not known, and, hence,
13 it deserves further study.

14 Finally, the role that changes in allocation of resources play in plant response to O₃ is now
15 better understood. Most studies have shown that O₃ decreases allocation of photosynthate to
16 roots. In some cases, allocation to leaf production has increased. Whether these changes are
17 driven entirely by changes in carbohydrate availability or are controlled by other factors (e.g.,
18 hormones) is not known. Physiological effects within the leaves inhibit photosynthesis; alter the
19 assimilation of photosynthate and shift its allocation patterns; and can lead to reduced biomass
20 production, growth, and yield (U.S. Environmental Protection Agency, 1986, 1996).

21 The major problem facing researchers trying to predict long-term O₃ effects on plants is
22 determining how plants integrate the responses to O₃ exposures into the overall naturally
23 occurring responses to environmental stressors. Little is now known about how plant responses
24 to O₃ exposures change with increasing age and size, but this information is crucial to predicting
25 the long-term consequence of O₃ exposure in forested ecosystems.

26 This section focuses on reactions of O₃ within cells and cellular tissue, in order to explain
27 known mechanisms that govern plant responses. The processes that occur at cell and tissue
28 levels within the leaf will be divided into several steps beginning with O₃ uptake and its initial
29 chemical transformations into a series of currently unknown, but suspected toxic, chemicals
30 (Figure AX9-2). The discussion will then focus sequentially upon various cell regions, their
31 general physiology, and the changes that may occur within a plant after O₃ exposure. This is

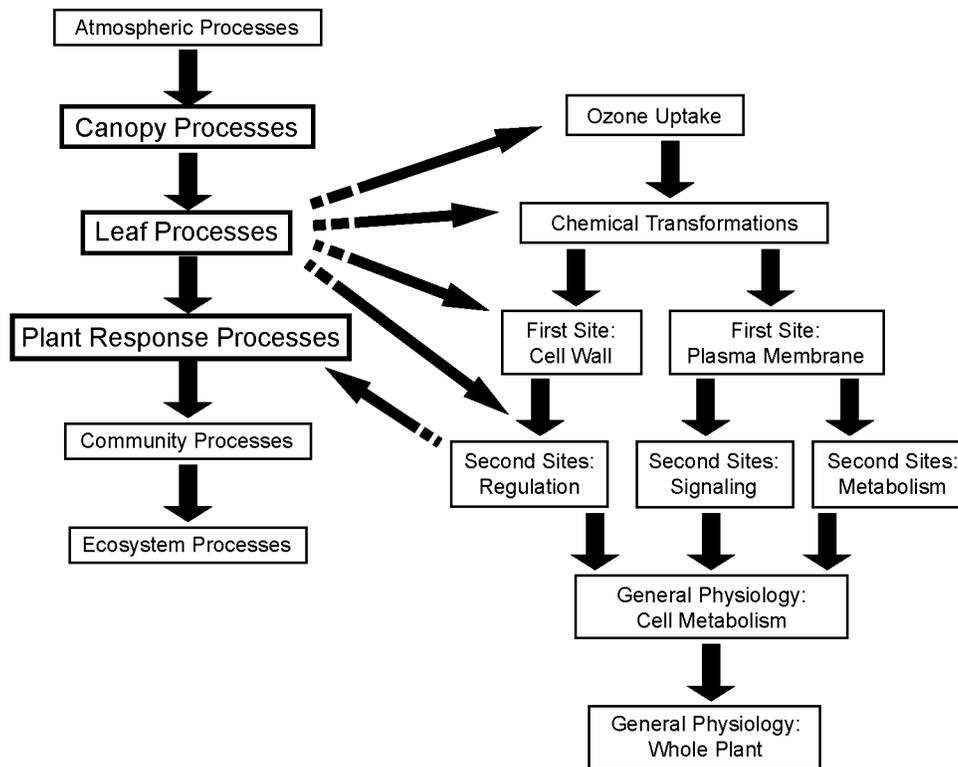


Figure AX9-2. Absorption and transformation of O₃ within the leaf. The varied processes are broken down in to smaller mechanistic steps that lead from uptake of atmospheric O₃ into the alterations which may occur within the individual plant. Each plant responds to the O₃ level and therefore interacts with the total ecological setting to generate an ecosystem response due to the O₃.

1 important because the varying responses of the different plant species in a community ultimately
 2 lead to an ecosystem response. Finally, a general summary is presented that discusses the known
 3 or suspected changes that occur within the whole plant.

4

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

6 Plants can survive O₃ stress through exclusion or tolerance mechanisms (Levitt, 1972)
 7 (Tingey and Taylor, 1982). Ozone may be excluded from tissues or cells via stomatal closure,
 8 by extracellular oxidants, or by membrane impermeability to O₃ or its products. Past
 9 investigations of O₃ injury have indicated that physiological and metabolic changes occur (Harris

1 and Bailey-Serres, 1994; Heath, 1988; Heath and Taylor, 1997; Reddy et al., 1993). Many of
2 these changes are likely initiated via gene expression. During the last decade, our understanding
3 of the cellular processes within plants has increased. Although the fundamental hypotheses
4 concerning O₃-induced changes in physiology have not changed, a more complete development
5 of the theories is now nearing possibility.

7 **AX9.2.2.1 Changes in Metabolic Processes: Current Theories**

8 The current hypotheses regarding the biochemical response to O₃ exposure revolve about
9 injury and its prevention. These are well discussed by Pell et al. (1997) and are listed below in
10 no order of importance. Although they are listed separately, some may be interlinked and related
11 to each other.

- 12 (1) Membrane Dysfunction. The membrane is altered by O₃, principally via protein
changes not involving the lipid portions of the membrane (except at extremely high
levels of O₃). 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 Ca²⁺/K⁺/H⁺ are the primary
suspects.
- 13 (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.
- 14 (3) Stress Ethylene Interactions. Visible injury is caused by the interaction of O₃ 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.
- 15 (4) Impairment of Photosynthesis. A product of O₃ (and less probably, O₃ 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 CO₂ 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 CO₂ fixation.
In any case, the response of the stomata to the current environment does not promote
efficient photosynthesis.

- 1 (5) Translocation Disruption. One of the biochemical systems most sensitive to O₃
exposure is the translocation of sugars, such that even a mild exposure inhibits the
translocation of carbohydrate (Grantz and Farrar, 1999, 2000).
- 2 (6) General Impairment/Disruption of Varied Pathways of Metabolism. This is the
oldest and most vague concept of how O₃ 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).

3 The latter two theories can be restated as a loss of productivity with three possible
4 somewhat-independent causes: (a) a reduced production of the basic building blocks of growth
5 and, hence, a slowing of growth in at least one organ; (b) a reduced ability to reproduce, leading
6 to a decreased production of viable seeds or of fruits and nuts; and (c) a decreased ability to
7 mount a defense against pathogens or insects, leading to weaker plants, which are more liable to
8 be overcome by other stresses. It is important to separate out effects that may be detrimental or
9 disfiguring, such as the production of visible injury, but which have not been shown to lead
10 directly to a loss of productivity due to possible compensation by the remaining tissue.

11 **AX9.2.2.2 Modifications of Plant Physiological Processes**

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

19 The high levels of O₃ used for some investigations do not automatically invalidate the
20 results obtained in those studies. Typically when a new hypothesis is being investigated,
21 extreme levels of the toxicant are used to determine its effects clearly. The older studies that
22 used concentrations as high 1 ppm, an extreme level, helped to define current studies. Later
23 experiments have used concentrations nearer ambient levels. Many of the current studies on
24 physiology use exposures between 0.15 and 0.25 ppm, which though higher than ambient levels
25 in some areas of the country, bypass confounding changes but allow for rapid experiments.
26

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

16 **AX9.2.3 Ozone Uptake by Leaves**

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

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

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

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

$$j = g(C_o - C_i) \quad (\text{AX9-1})$$

19
20
21
22 where the flux, j , into the internal space of a leaf is related to the conductance, g , through the
23 boundary layer and stomata and the gradient of concentration of gas from the outside, C_o ,
24 inward, C_i . This formulation has been used for years for both water and CO₂ (Figure AX9-4),
25 and for regions of varied CO₂ concentration that correspond to C_o (CO₂ of the atmospheric air,
26 below the leaf proper) and C_i (CO₂ near the leaf’s spongy mesophyll cells) (Ball, 1987; Farquhar
27 and Sharkey, 1982).
28

29 In the past, the internal concentration of O₃ has been assumed to be zero (Laisk et al.,
30 1989), due to early studies that found that virtually no O₃ could pass through a leaf. That was
31 expected because O₃ is extremely reactive with cellular biochemicals. If the assumption that the
32 internal concentration zero is correct, then the effective delivery rate for O₃ is given as $g \times C_o$,

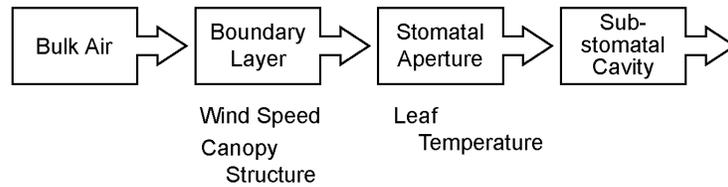


Figure AX9-3. The uptake of O_3 into the leaf. Each of the individual concentration layers of O_3 represents a different process of movement and of plant/microenvironmental interaction. This figure leads into Table 9-6, in which the amounts of O_3 along the pathway are calculated.

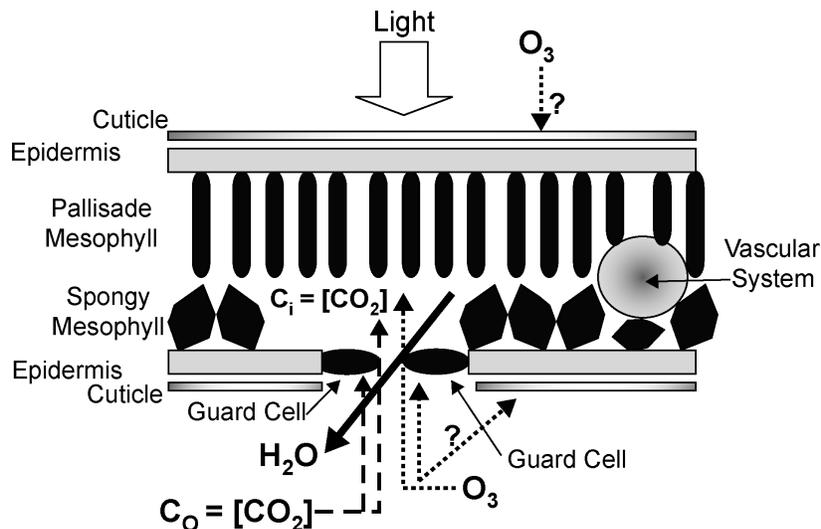


Figure AX9-4. The microarchitecture of a dicot leaf. While details among species vary, the general overview remains the same. Light that drives photosynthesis generally falls upon the upper (adaxial) leaf surface. Carbon dioxide and O_3 enters through the stomata on the lower (abaxial) leaf surface, while water vapor exits through the stomata (transpiration).

1 with stomatal conductance being the major regulatory control (Amiro et al., 1984; Taylor et al.,
 2 1982). However, a recent study by Moldau and Bichele (2002) indicated that the internal O_3
 3 concentration may not be zero as previous assumed. Moldau and Bichele (2002) permitted
 4 leaves of *Phaseolus vulgaris* L., which have stomata on both upper and lower leaf surfaces, to

1 take up O₃ at a high rate for 3 to 5 min. Exposure of the lower leaf surface resulted in up to 5%
2 of the O₃ that was taken up to be diffused through the leaf, emerging from the stomata on the
3 upper surface. This suggested the presence of above-zero concentrations of O₃ in the
4 intercellular leaf air spaces. The descriptive calculations and plots of Moldau and Bichele
5 (2002) indicate that the rise in internal O₃ level (for both concentrations of external O₃) within
6 the first few minutes of exposure is due to its reaction with an antioxidant, most probably
7 absorbate, within the apoplastic space of the leaf (Figure AX9-5). The rate of rise is probably
8 due to more complete penetration of O₃ with a concurrent depletion of the external antioxidant.
9 The rise peaks at about 2 min for 0.88 ppm and 3 min for 0.34 ppm and then falls to a lower
10 level. This may be due to a replenishment of the antioxidant. The authors saw no injury to the
11 plasmalemma (as measured by penetration of a dye) and no change in the stomatal conductance
12 for the lower concentration of O₃ (Moldau and Bichele, 2002). The higher level (0.88 ppm)
13 caused the plasmalemma of the mesophyll cells to pass a dye, and a slight decline in stomatal
14 conductance resulted at about 2.5 minutes. These data suggest that the antioxidant hypothesis is
15 correct.

16 Gaseous pollutants flow from the substomatal cavity within the leaf through the cell wall
17 into the cell. It is suspected that the internal concentration of the pollutant is not uniform within
18 the cavity (Taylor, and Hanson, 1992). From within the wall, an equilibrium between the gas
19 and aqueous phase must occur at the interface where the gaseous species dissolve into the water
20 according to Henry's Law (Heath, 1980, 1987; Wellburn, 1990). It is important to understand
21 exactly how much O₃ could move into the tissue of the leaves. Calculations in Table AX9-6
22 give an indication of the amount of O₃ which may end up near the surface of cells within the
23 leaf. The calculation is done for a standard temperature (25 °C), an ambient concentration of O₃
24 (0.10 ppm), and for nonspecific leaves. For example, 0.3 ppm would be the same general
25 numbers but multiplied by 3. Similarly for more closed stomata, the value of 1.0 cm/s
26 (equivalent to about 400 μmole⁻²-leaf area s⁻¹) for a conductance would be reduced and the
27 smaller values would lead to a smaller amount of O₃ moving into the tissue. Nonetheless, these
28 values give some indication of what sort of chemical concentration can be expected. Under
29 these conditions, a delivery rate of O₃ into the substomatal cavity near the spongy mesophyll
30 tissue of about 0.42 nmol/(L hr) appears to be reasonable.

31

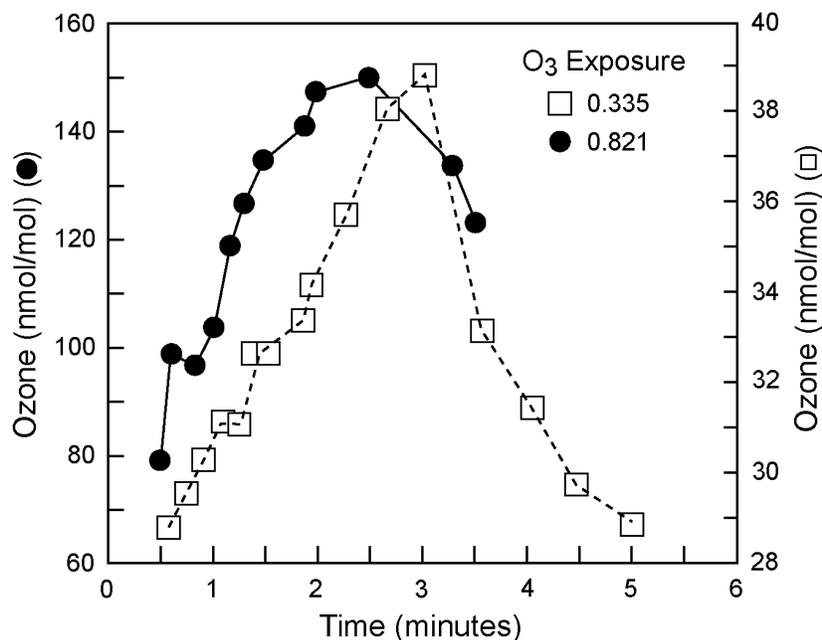


Figure AX9-5. The change in the O₃ concentration inside a leaf with time. Data are from O₃ exposures at two different concentrations.

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

1 Process 2. Ozone diffuses into the leaf air spaces and reacts either with varied biochemical
 2 compounds that are exposed to the air (path 1) or is solubilized into the water lining the cell wall
 3 of the air spaces (path 2). As shown in Figure AX9-6, each reaction has the possibility of
 4 transforming O₃ into another chemical species (a toxicant) which, in turn, may react with other
 5 chemical species and lead to a cascade of reactions.

6 Within the stomata, gases react with the water at the cell's surface and generate new
 7 species with the components within the cell wall region. The possible varied pathways are
 8 depicted in Figure AX9-7. Although these chemical reactions are poorly understood, some of
 9 the fundamentals are known (Heath, 1987, 1988; Wellburn, 1990). Ozone reacts with organic
 10 molecules at the double bonds to form carbonyl groups and, under certain circumstances,
 11 generates peroxides, such as hydrogen peroxides (H₂O₂), superoxide (O₂⁻) and its protonated
 12 form (HO₂^{*}), hydroxyl radicals (HO^{*}), and peroxy radicals (HO₂^{*}). Other chemicals present in

Table AX9-6. The Flow of Ozone into a Leaf and Possible Reactions

The level of O₃ 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.

DESCRIPTION

VALUES

The atmospheric level of O₃ is given as:

$$O_{3a} = 0.1 \text{ ppm}$$

For an air temperature of:

$$T_a = 25 \text{ }^\circ\text{C}$$

The perfect gas law ($pV = nRT$) is used to convert the O₃ level into standard mks. Further, the volume for a mole of gas ($V_o = 22400 \text{ m}^3$) will be used, from the perfect gas law with $R = 8.3144$

Thus, the concentration of O₃ within the atmosphere is:

$$C_{O_3} = \frac{O_{3a} \times 10^{-6} \cdot (T_a + 273.18)}{V_o \times 273.18}$$

$$C_{O_3} = 4.873 \times 10^{-12} \text{ moles/m}^3$$

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:

$$gs_{wv} = 1 \text{ cm/s}$$

The amount of O₃ that will penetrate inside the leaf (for a typical concentration of nearly zero inside the leaf), is:

$$O_{3L} = \sqrt{\frac{18}{48} \frac{gs_{wv}}{100}} C_{O_3}$$

$$O_{3L} = 2.984 \times 10^{-14} \text{ mol/(m}^2 \text{ s)}$$

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:

$$FW_L = 30 \text{ mg/cm}^2$$

Thus, the square surface area of the leaf will translate into water space (for concentration of chemicals), as:

$$Ar_L = \frac{FW_L \times 0.85}{100}$$

$$Ar_L = 0.255 \text{ L/m}^2$$

The maximum amount of toxic compound that will be generated, assuming all the O₃ 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 O₃ is present (and no back reactions occur) for one hour (short but typical units of exposure).

$$O_{3Lc} = (O_{3L} / Ar_L) \times 3600$$

$$O_{3Lc} = 4.21 \times 10^{-10} \text{ mol/(L hr)}$$

Table AX9-6 (cont'd). The Flow of Ozone into a Leaf and Possible Reactions

Thus, the maximum amount of toxic chemicals generated per hour in a leaf would be:

$$O_{3Lc} = 0.42 \text{ nmol/(L hr)}$$

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.

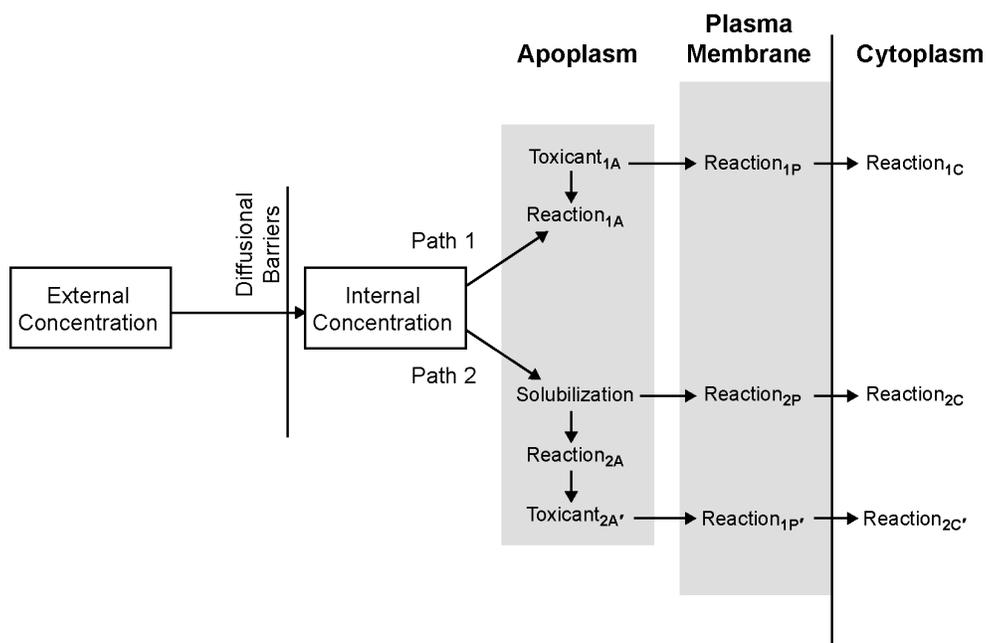


Figure AX9-6. Possible transformations of O_3 within a leaf.

1 the water phase can lead to many other oxygenated moieties (Figure AX9-6). Each of the steps
 2 is generally pH dependent (Jans and Hoigne, 2000; Walcek et al., 1997).

3 Sulfhydryls are particularly easy targets, with the formation of disulfide bridges or sulfones
 4 (Mudd and Kozlowski, 1975). In water, the reactions become more confusing, but some
 5 products have been described by Heath and Castillo (1987), such as H_2O_2 , HO^* , and O_2^-
 6 (Figure AX9-7). Effective detoxification reactions can occur here via antioxidant metabolites
 7 and enzymes such as ascorbate, glutathione (GSH), and superoxide dismutase (SOD) if they are
 8 present at high enough concentrations (Castillo et al., 1987; Matters and Scandalios, 1987).

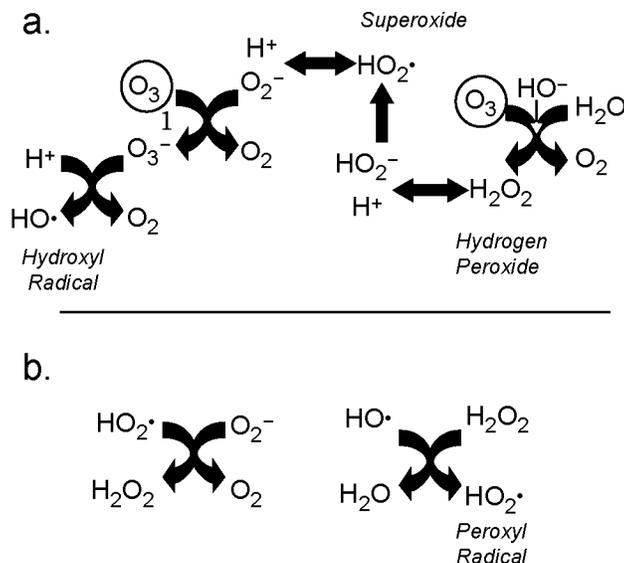


Figure AX9-7. Possible reactions of O₃ within water. (a) Ozone reacts at the double bonds to form carbonyl groups. (b) Under certain circumstances, peroxides are generated.

1 If the levels are low, it is believed that stimulation of their production is a response to O₃, albeit a
 2 slow one (Harris and Bailey-Serres, 1994). Certainly it is possible that chemical modification of
 3 wall-specific biochemicals (Castillo et al., 1987) such as glucan synthase (Ordin et al., 1969) and
 4 diamine oxidase (Peters et al., 1988) occurs.

5

6 *Process 3. Movement of reaction product(s) into and enzymatic or chemical*

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

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

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

11 Ozone can react with many compounds within the substomatal cavity of the leaf¹ to
12 produce a variety of oxidizing and toxic chemicals. Some of the possible reactions that will
13 generate H₂O₂, HO•, and SO₂⁻, as well as charged O₃ intermediates, are indicated in Figure
14 AX9-8. Many of these complex reactions have been studied within water solutions through
15 research of O₃-induced water purification and are very dependent upon solutes present with the
16 solutions, including H⁺ (see Von Gunten [2003]). An important point is that in alkaline
17 media, O₃ forms H₂O₂, but in acid media, O₃ is relatively stable in the absence of free metal ions.

18 The rates of reaction of O₃ with several important compounds, including those with a
19 double bond, the so-called Crigee Mechanism shown in Figure AX9-8, can be calculated from
20 the reaction coefficient as given by Atkinson (1990) (Table AX9-7). The double bond of the
21 ascorbate molecule is particularly sensitive to O₃ attack. Because of the ring formation of the
22 ascorbate molecule, an unstable ozonide product is formed, which then accelerates the breakage
23 of the double bond, leading to the formation of two products. These products are relatively
24 unstable and can lead to further reactions not shown in Figure AX9-8. The rates of reactions can
25 be calculated (Heath, 1987). At a local concentration of 25 μM O₃, it would take 5000 s
26 (83 min) for all of the O₃ to react if there was no further flow of O₃. Clearly, O₃ does not react
27 rapidly with the compounds in Table AX9-7 and, although some of the products would be
28 formed through the Crigee Mechanism (see Figure AX9-8a), they would be low in

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.

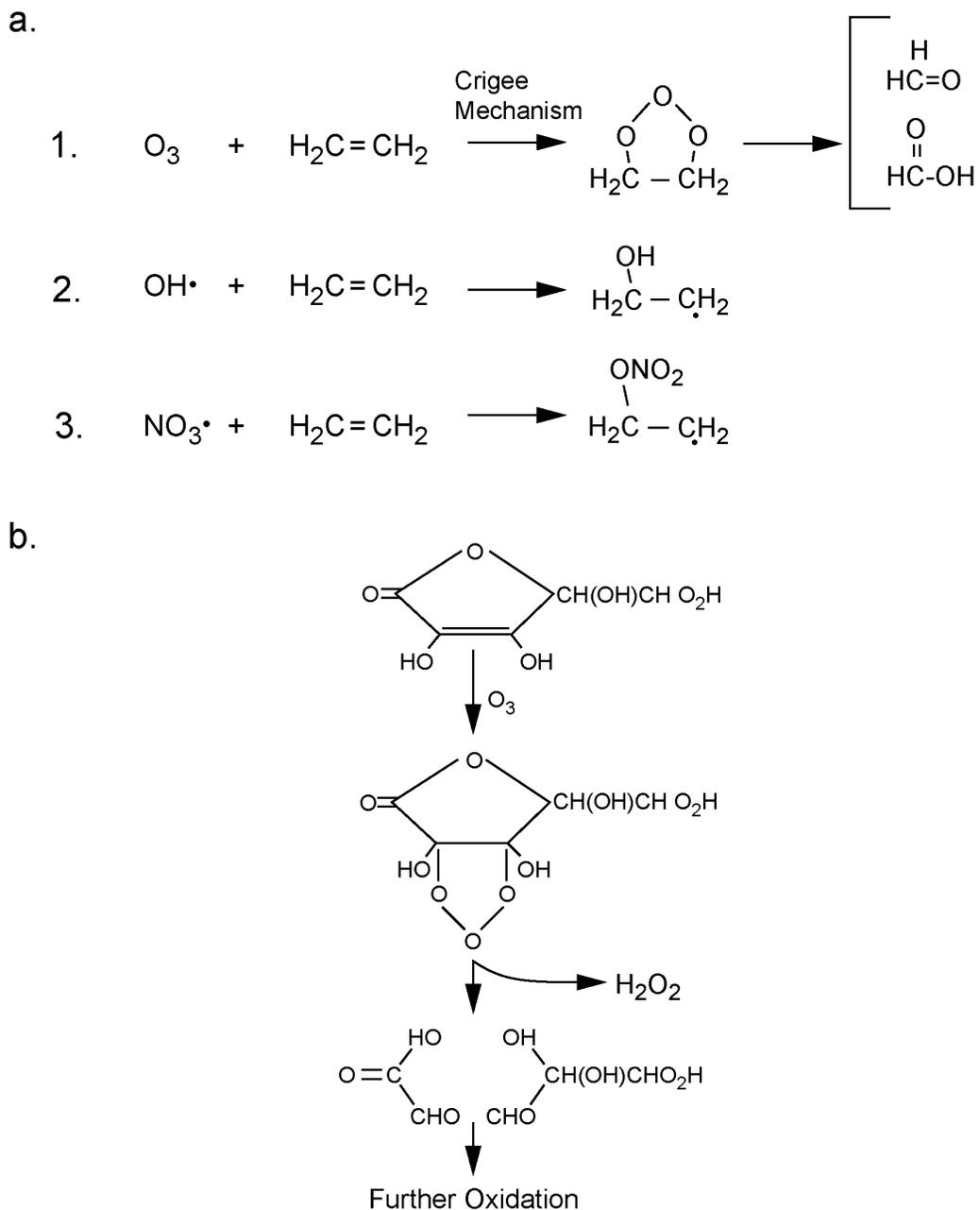


Figure AX9-8a,b. The Crige mechanism of O_3 attack of a double bond. (a) The typical Crige mechanism is shown in which several reactions paths from the initial product is shown. (b) Typical reaction of ascorbic acid with O_3 .

Source: Adapted from Mudd (1996).

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 O₃ in the air stream within the leaf (localized concentration of about 25 mM, see Table AX9-6).

Compound	$\times 10^{-18} \text{ cm}^3/\text{molecules s}^{-1}$	Rate coefficient (L/mole s ⁻¹)	Rate of reaction (M/s)
Ethane	1.7	1.02×10^3	4.3×10^{-11}
Propene	11.3	6.80×10^3	2.8×10^{-10}
1-butene	11	5.91×10^3	2.5×10^{-10}
trans-2 Butene	200	1.20×10^5	5.0×10^{-9}
α -pinene	85	5.12×10^4	2.1×10^{-9}

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

Reactions	Rate constants
(1) $\text{O}_3 + \text{OH}^- + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 + \text{OH}^-$	$k_1 = 3.67 \times 10 \text{ mole}^{-1} \text{ L s}^{-1}$
(2) $\text{O}_3 + \text{O}_2^- \rightarrow \text{HO}^\cdot + 2 \text{O}_2 + \text{OH}^-$	$k_2 = 1.26 \times 10^9 \text{ mole}^{-1} \text{ L s}^{-1}$
(3) $\text{O}_3 + \text{HO}_2^- \rightarrow \text{HO}^\cdot + \text{O}_2^- + \text{O}_2$	$k_3 = 2.09 \times 10^6 \text{ mole}^{-1} \text{ L s}^{-1}$

[c] Possible Concentrations of Other Oxidative Species. Table from Heath (1987). Based upon 100 ppm O₃ in gas stream.

Species	Concentration (M)		Molecules within wall	
	pH 7	pH 9		
Superoxide Radical (O ₂ ^{•-})	8.75×10^{-15}	1×10^{-12}	5.5×10^{-6}	6.3×10^{-4}
Ozone Radical	4.16×10^{-15}	5×10^{-14}		
Protonated O ₃ radical (HO ₃ [•])	1.48×10^{-16}	1×10^{-18}		

Number of molecules within apoplastic space of (10⁻¹² L) at 0.1 ppm O₃.

1 concentration². While other radicals, such as the hydroxyl radical (see Figure AX9-8b) can
 2 attack double bonds, the products differ. Of particular note for later discussion, is the reaction
 3 of O₃ with ascorbate (see Mudd (1996) (see Figure AX9-8b), which will cleave the double bond
 4 in the ring. Unfortunately, little work has been done to characterize possible products within the
 5 leaf (but see next section).

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.

1 In a paper discussing the stability and reactivity of O₃ in the pulmonary air/tissue
2 boundary, Pryor (1992) calculated that O₃ has a half-life of about 7 × 10⁻⁸ s in a bilayer.
3 However, the transit time through the lung lining fluid layer is about 2 × 10⁻⁶ s, based upon a
4 reasonable estimate for the diffusion of O₃. This means that O₃ would suffer nearly 29 half-
5 lives³ in passage through the layer, reducing it to about 3 × 10⁻⁹ of the original concentration —
6 zero for all practical aspects. In the same publication, Pryor points out that any sulfhydryl or
7 ascorbate would interact strongly with O₃, further reducing its net concentration. The reactivity
8 of cysteine is 10⁹, while the reactivity of tryptophan, methionine, polyunsaturated fatty acids,
9 and tyrosine is about 2 × 10⁶ and that of phenylalanine is only 10³. These numbers are similar to
10 what has been found for O₃ reactivity with amino acids and proteins in aqueous solutions.
11 In glycophorin (Banerjee and Mudd, 1992) and cytochrome C (Mudd et al., 1997b; Mudd et al.,
12 1996) in aqueous solutions, only the methionine was oxidized by O₃, producing sulfoxide.
13 In other proteins lacking methionine, tryptophans were oxidized only if they were in an exposed
14 position on the surface of the proteins (Mudd et al., 1997b). Treatment of red blood cell ghosts
15 with O₃, oxidized peripheral proteins of the plasma membrane before it oxidized lipids (Mudd
16 et al., 1997a).

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

19 While Mehlhorn et al. (1990) are often thought to have shown that free radicals were
20 formed in plant leaves under O₃ exposure, careful reading of that paper clearly shows that there
21 was no real evidence of free radicals induced by O₃. Living tissues have many free radical
22 signals, making it difficult to observe changes in free radicals. Further, the work of Grimes et al.
23 (1983) has also been cited as showing the presence of free radicals in living tissues due to O₃
24 exposure; however, no radical signals were found unless certain organic acids (e.g., caffeic acid)
25 were added to the tissue with the O₃ exposure. They used the radical trap TMPO
26 (tetramethylphrrolise 1-oxide) which reacts with many types of free radicals to form a stable
27 radical that can be used to “trap” or increase the amount of radical present (see Figure AX9-9a).
28 Ozone would directly react with this trap only if it were bubbled into the solution, not passed
29 over the top of the solution. In the presence of sorbitol or caffeic acid, the trap would indicate

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.

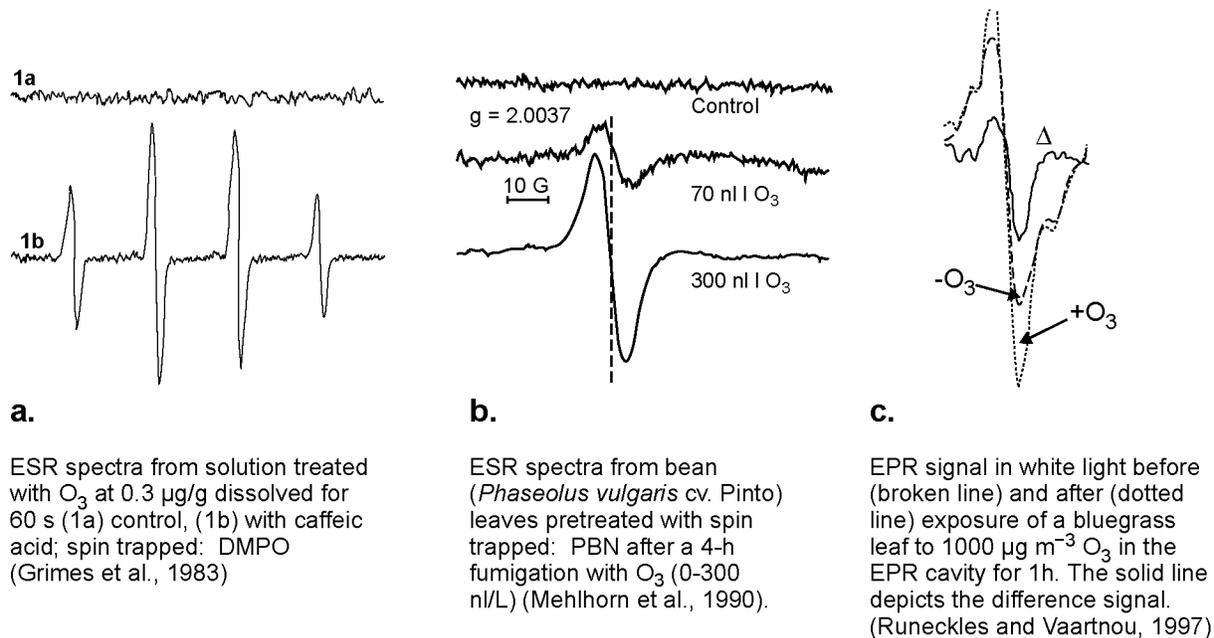


Figure AX9-9. Varied ESR radicals, trapped and not, generated by O_3 under somewhat physiological conditions. (a) The generation of a DMPO-trapped radical with caffeic acid in water solution (Grimes et al., 1983). (b) The generation of a DMPO-trapped radical within bean (*Phaseolus vulgaris* cv. Pinto) exposed to 70 nL/L O_3 for 4 h. The lower trace is the ESR signal produced with 300 nL/L O_3 (Mehlhorn et al., 1990). (c) The EPR signal produced within a bluegrass leaf exposed to $1000 \mu\text{g m}^{-3}$ of 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.

1 the presence of OH radical, which would mean that $O_3 \rightarrow HO^\bullet$. Superoxide dismutase, catalase,
 2 or EDU had no effect upon this signal, suggesting O_2^- and H_2O_2 were not involved in the above
 3 sequence. Both O_3 and O_3 plus caffeic acid had no effect upon the protoplasts' intactness or
 4 viability. Thus, 10^{-5} M HO^\bullet and/or 0.30 to 0.40 ppm O_3 did not react with the cell membrane.
 5 They found no signal in normal cells after subjecting the leaf to O_3 and concluded that the
 6 radicals were produced via a concerted mechanism with the acid. This does not fit with the
 7 mechanism postulated by Mehlhorn et al. (1991), which involved a reaction of wound-induced
 8 ethylene and O_3 at the wall level to generate some free radicals.

1 The hypothesis that the production of wound-induced ethylene by O₃ exposure and its
2 reaction with O₃ would result in the production of radicals was tested by Mehlhorn et al. (1990),
3 using electron paramagnetic resonance spectroscopy. After 4 h of 300 ppb, an EPR signal of a
4 compound was detected which resembled a butonyl radical (Figure AX9-9b [Mehlhorn et al.,
5 1990]). Using 70 ppb, the signal was reduced by about one third that of an ethyl radical⁴, leading
6 to injury. However, the spraying of the plant with 1-aminoethoxyvinyl-Gly (AVG), which
7 reduces the production of ethylene and visible injury, had no effect upon the EPR signal,
8 suggesting that the radical is not a direct sequela to visible injury.

9 Runeckles and Vaartnou (1997) (Figure AX9-9c) discovered a signal by subtracting other
10 EPR signals of the leaf, which seemed to be due to an O₃ reaction with plant material, using
11 0.48 ppm O₃. This difference signal looked very much like O₂⁻. At a lower concentration, they
12 observed that this signal still occurred but accumulated more slowly. Both bluegrass and
13 ryegrass leaves seemed to saturate after about 5 h of exposure at 22 to 28 units of signal, while
14 radish leaves reached a maximum of 7 units at 3 h and then declined. The problem, which is
15 typical of any of these methods, was that the detached leaf had to be rolled and placed into the
16 EPR detection cavity. Reichenauer et al. (1998) also detected an undefined free radical signal
17 that seemed to be related to a Mn(II) spectrum. The Nandu and Perlo cultivars of wheat were
18 more sensitive to O₃ than Extradur (according to growth rate and closure of stomata under an O₃
19 exposure of 80 ppb for 8 h/day, 7 days/week over 100 days), and these more sensitive cultivars
20 had a greater, but insignificant (P = 15%), EPR signal. Thus, data showing any production of a
21 free radical must be approached with some skepticism.

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

⁴The reaction would be: O₃ + H₂C = CH₂ → varied C-1 compounds, due to double bond cleavage, at a
rate constant of 1.7 × 10⁻¹⁸ cm³/molecule sec = 1.02 × 10³ 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 × 10⁻¹² cm³ molecule⁻¹ s⁻¹,
or 10⁶ × faster.

1 **AX9.2.3.3 Products of Ozone**

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

7 8 ***Hydrogen Peroxide***

9 Hydrogen peroxide, until recently was thought to be purely a toxic compound for cells.
10 However, it is now clear that it functions as a signaling molecule in plants and mediates
11 responses to abiotic and biotic stressors (Figure AX9-10). Generation of H₂O₂ is increased in
12 response to various stressors, implicating it as a key factor mediating the phenomena of
13 acclimation and cross tolerance, in which exposure to one stressor can induce tolerance of
14 subsequent exposure to the same or different stresses (Neill et al., 2002). The signaling response
15 to attack by invading pathogens using H₂O₂ has been described (Mehdy, 1994; Simon-Plas et al.,
16 1997). The reactions leading to hypersensitive cell death are caused by a pathogen recognition
17 step (Figure AX9-10a), probably due to the plant cell wall releasing oligosaccharides in response
18 to the pathogen enzymatically breaking down the cell wall to penetrate it. A feed-forward step
19 in which H₂O₂ increases the level of benzoic acid leads to the activation of the hydroxylase step
20 in the production of salicylic acid and to a feedback step in which the salicylic acid increases the
21 production of H₂O₂ (Leon et al., 1995).

22 An elicitor, e.g., a bacterial or fungal pathogen, induces a cascade of reactions within a cell
23 (Figure AX9-10b). Some of the lipid reactions are thought to be due to the opening of the Ca²⁺
24 channels and the alkalination of the cell wall region. The oxidative burst due to H₂O₂ production
25 is believed to lead to the transformation of a small population of lipids into jasmonic acid, which
26 is a secondary messenger.

27 Hydrogen peroxide also has an oxidative role in lignification (Schopfer, 1994). In the
28 interaction of lignification and the beginning processes of hypersensitivity, pectinase produced
29 by the pathogen disrupts pectin and dissolves the cell wall. Fragments of the dissolved cell wall
30 trigger an increase in the transcription of peroxidases within the remaining cell wall, leading to

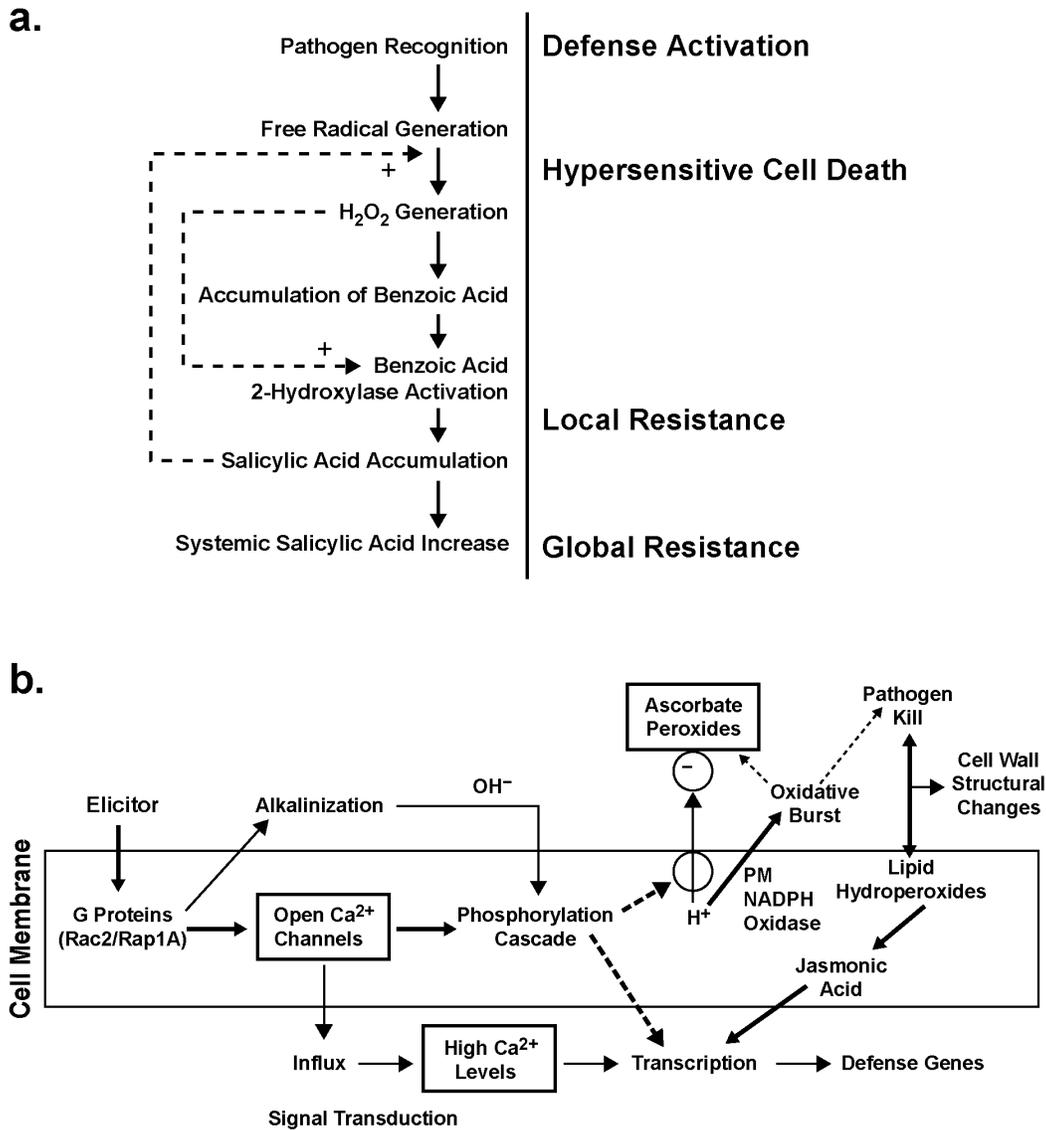


Figure AX9-10. Pathogen-Induced Hypersensitivity. (a) The reactions leading to hypersensitive cell death and the formation of a global response of salicylic acid. (b) The cascade of the elicitor-induced reactions within the cell.

1 lignification, which is a cross-linking of the cell wall that does not use pectin. This prevents
 2 further pathogen disruption of the wall and reduces its further entry into the plant cell.

3 It is believed that the first species generated through a one-electron reduction of molecular
 4 oxygen is SO_2^- . That generation is carried out using a cytochrome b_6 by the NAD(P)H oxidase

1 located on the cell membrane (Auh and Murphy, 1995). In the acid region of the cell wall, SO_2^-
2 is converted by a protonation and dismutation to H_2O_2 . The induced oxidative burst is believed
3 to play a role in stimulating the Cl^- and K^+ efflux, generating an alkalinization of the
4 extracellular space (Cazale et al., 1998). In the wall region, H_2O_2 is not especially toxic, as no
5 necrosis was reported in tobacco when 500 mM peroxide was infiltrated into the leaf tissue.
6 However, the production of salicylic acid and benzoic 2-hydroxylase can be induced with only
7 30 and 0.3 mM H_2O_2 , respectively, indicating some metabolic signaling (Leon et al., 1995).
8 On the other hand, 1M H_2O_2 infiltrated into soybean will generate lipid peroxidation after 1 h
9 with a peroxidation rate of 15 nmol/g-FW h (Degousee et al., 1994). Cells react to the system⁵
10 and generate peroxide scavenging compounds within 1.5 to 2 hours, which appear to “mop up”
11 the excess H_2O_2 (Baker et al., 1995).

12 After O_3 exposure in birch, H_2O_2 has been found in the wall (Pellinen et al., 1999). By
13 using CeCl_2 as a cellular stain for H_2O_2 (as a cerium perhydroxide precipitate), Liu et al. (1995)
14 observed a gradual development of stain after 8 h of O_3 exposure (at 150 ppb). After 2 h
15 exposure, H_2O_2 stain was visible on the surfaces of both sets of mesophyll cells. Accumulation
16 of H_2O_2 stain continued for 16 h after exposure, suggesting a triggered-reaction rather than O_3
17 decomposition itself. H_2O_2 stain was present in the mitochondria, peroxisomes, and cytoplasm
18 but not in the chloroplast. If methyl viologen (MV) was given to the leaves and then the leaves
19 were exposed to light, H_2O_2 stain could be observed within the chloroplast. This indicated that
20 the stain worked within the chloroplast if H_2O_2 were generated by the Mehler reaction
21 ($\text{MV} + \text{O}_2^-$). Thus, apparently, for birch, O_3 exposure does not generate excess H_2O_2 within the
22 chloroplast. Furthermore, these sets of experiments indicate that O_3 per se does not generate the
23 H_2O_2 , but rather triggers stress-related H_2O_2 formation similar to what occurs in a pathogen
24 attack (the Reactive Oxidative Species or ROS reaction).

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

⁵Soybean suspension cells were inoculated with *Pseudomonas syringae* pv *syringae*, which generate an active oxygen response. Light emission by luminol, reacting with H_2O_2 , was the assay for the peroxide.

1 H₂O₂ has been linked to the hormone ABA-induced closure of the stomata by activating the
2 calcium influx in guard cells (Pel et al., 2000). The addition of H₂O₂ at a level of only 5 mM to a
3 guard cell preparation will cause a dramatic increase (ca. 9×) in electrical current at the
4 hyperpolarizing potential of -200 mV. Amounts as low 50 mM H₂O₂ will cause a less, but still
5 sizable, increase. Membrane stability is unaffected by the H₂O₂ and the activation of the channel
6 requires only about 2 to 3 minutes. Pel et al. (2000) also found that ABA induced the production
7 of H₂O₂ through ROS accumulation (also see Zhang et al. [2001]).

8 Certain levels of ABA within the leaf lead to stomatal closure. The inactivation of a
9 phospho-tyrosine-specific protein phosphatase (ABI2) is an inhibitor of stomatal opening
10 induced by ABA but that enzyme is inhibited by H₂O₂ (Meinhard et al., 2002). This means that
11 H₂O₂ shifts the sensitivity of the stomatal opening to ABA (Figure AX9-11), making the
12 stomatal complex more sensitive to ABA. Thus, for a given level of ABA present in the guard
13 cell complex due to environmental factors (e.g., low humidity, high air temperature, or low soil
14 water potential), the generation of H₂O₂ would (by inhibiting ABI2) induce a closure of the
15 stomata by increasing the sensitivity of the guard cells to ABA. In the past, it has been difficult
16 to understand why O₃ would often decrease conductance in some cases, but not always (Heath,
17 1994b). This interaction between H₂O₂ and ABA could help understand this complexity.

18 19 ***Ethylene Reactivity***

20 Ethylene (ET) is produced when plants are subjected to biotic stressors, e.g., attacks by
21 insects, fungi, and bacteria or abiotic stressors such as wounding or environmental stressors such
22 as heat, cold, or oxidative stress and O₃. If an O₃ stress has induced a wounding response with
23 ET release, then ET within the substomatal cavity could react with O₃, generating some
24 relatively noxious chemicals (see Figure AX9-6). The relationship between O₃ injury and
25 wounding is supported by the observation by Mehlhorn et al. (1991) that an inhibitor of ET
26 formation, AVG (an inhibitor of ACC synthase, a committed step to ET production), would
27 block ET formation and inhibit visible injury. Other studies with polyamine (which is closely
28 linked to ET production), including those of Ormrod and Beckerson (1986) who fed polyamines
29 to the transpirational stream and prevented visible injury, suggested a close involvement of both
30 pathways to the production of visible injury. Both the lack of ET production and an increased
31 level of polyamines slowed or prevented visible injury.

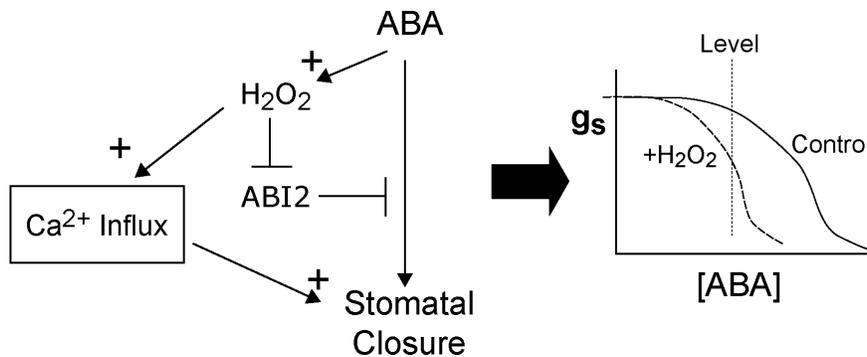


Figure AX9-11. The interaction of H₂O₂ and Ca²⁺ movements with ABA-induced stomatal closure. It is well known that certain levels of ABA within the leaf lead to closure of the stomata within the leaf. That level, however, can be shifted to make closure more or less sensitive to a given level of ABA. Recently it has been shown that H₂O₂ (externally or produced by the plant) within the cell wall region can shift that sensitivity. Here ABA stimulates the production of H₂O₂, which in turn increases the rate of Ca²⁺ moving from the wall region into the cytoplasm. That shift in internal Ca²⁺ 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.

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

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

8 More recent studies, however, indicate that O₃ responses resemble components of the
 9 hypersensitive response (HR) observed in incompatible plant-pathogen interactions
 10 (Sandermann, 1998). The similarity to the HR response may be related to the occurrence of
 11 ROS in the apoplast. The O₃-derived ROS apparently trigger an oxidative burst in the affected
 12 cells by an as yet unknown mechanism. An oxidative burst is similar to one of the earliest

1 responses of plants to microbial pathogens and is an integral component in HR-related cell death
2 (Overmyer et al., 2000).

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

11 12 ***Ethylene-Interaction with Injury and Conductance***

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

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

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

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

16 17 **AX9.2.3.4 Antioxidants Within the Apoplastic Space**

18 The first line of defense against O₃ is a closure of the stomata to exclude its uptake. This is
19 counterproductive for efficient photosynthesis, but some amount of closure limits the rate of O₃
20 deposition into the leaf tissue to allow for a secondary line of defense to detoxify the O₃. The
21 secondary line of defense involves a range of antioxidants, which are highly reactive to the types
22 of chemicals that can be generated by O₃. Several antioxidant proteins are stimulated by O₃ in
23 *Plumbagini folia*, including glutathione peroxidase (GSH-P_x), SOD, and catalase. The
24 timescales for changes in their levels vary: some rise rapidly, while others rise more slowly.
25 The pattern of changes in these particular proteins varies greatly among different species and
26 conditions.

27 28 ***Ascorbate Within the Cell Wall***

29 Most of the recent reports indicate that ascorbate within the cell wall is the real first line of
30 all defense. Ascorbate within the wall declines when the tissue is exposed to O₃ (Luwe et al.,

1 1993; Moldau, 1998). This decline appears to be closely linked to the amount of O₃ penetrating
2 the leaf tissue.

3 It has long been suspected that intracellular antioxidants play a role in preventing O₃-
4 induced injury to plant cells. Variation in the types of biochemical compounds present in the
5 apoplastic space can give rise to a multiplicity of reactions with O₃, but the predominant
6 biochemical species is ascorbate. Ascorbate is water soluble, present in the solution where O₃
7 can dissolve, and is highly reactive. Unfortunately, a variety of antioxidants are found
8 throughout the cell and any measurements of one particular type within the total leaf tissue can
9 give misleading results. For example, ascorbate is present within the cell wall, cytoplasm, and
10 chloroplasts (Burkey, 1999; Moldau, 1998); and ascorbate can move between the cytoplasm and
11 the cell wall with relative ease (Figure AX9-12) (Bichele et al., 2000). The total of all ascorbate
12 pools is measured when the tissue is ground and assayed. If the cell wall ascorbate concentration
13 drops by 50% due to O₃ exposure but all other tissue concentrations remain the same, the
14 measurement of the total loss is dependent upon the amount of ascorbate within the cell wall.
15 Turcsányi et al. (2000) showed that, compared to the concentration of apoplastic ascorbate, the
16 rest of the cells contained about 38 times as much. So a 50% loss of apoplastic ascorbate would
17 be converted into only 2 to 3% loss of the total ascorbate.

18 The ascorbate deficient *Arabidopsis thaliana* mutant has proven to be a powerful tool in
19 furthering the understanding of ascorbate biosynthesis in plants (Smirnoff et al., 2001). Three
20 classes of mutants were formed when *A. thaliana* seed was mutagenized with ethyl
21 methanesulfonate: (a) those deficient in SOD, (b) those that failed to accumulate more
22 antioxidant proteins upon increased O₃ exposure, and (c) those that were deficient (but not
23 depleted) in ascorbate. The low-ascorbate mutant type had 50 to 60% less ascorbate than the
24 wild type and displayed more foliar injury. This mutant is involved with the coding of the
25 GDP-D-Mannose pyrophosphorylase enzyme⁶ in the Smirnoff-Wheeler pathway for ascorbate
26 biosynthesis. Smirnoff et al. (2001) also suggested that other pathways can produce ascorbate
27 without relying upon the pyrophosphorylase step, but most probably at a slower through-put rate,
28 because any fully ascorbate-deficient mutant would be lethal, perhaps because of ascorbate use

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.

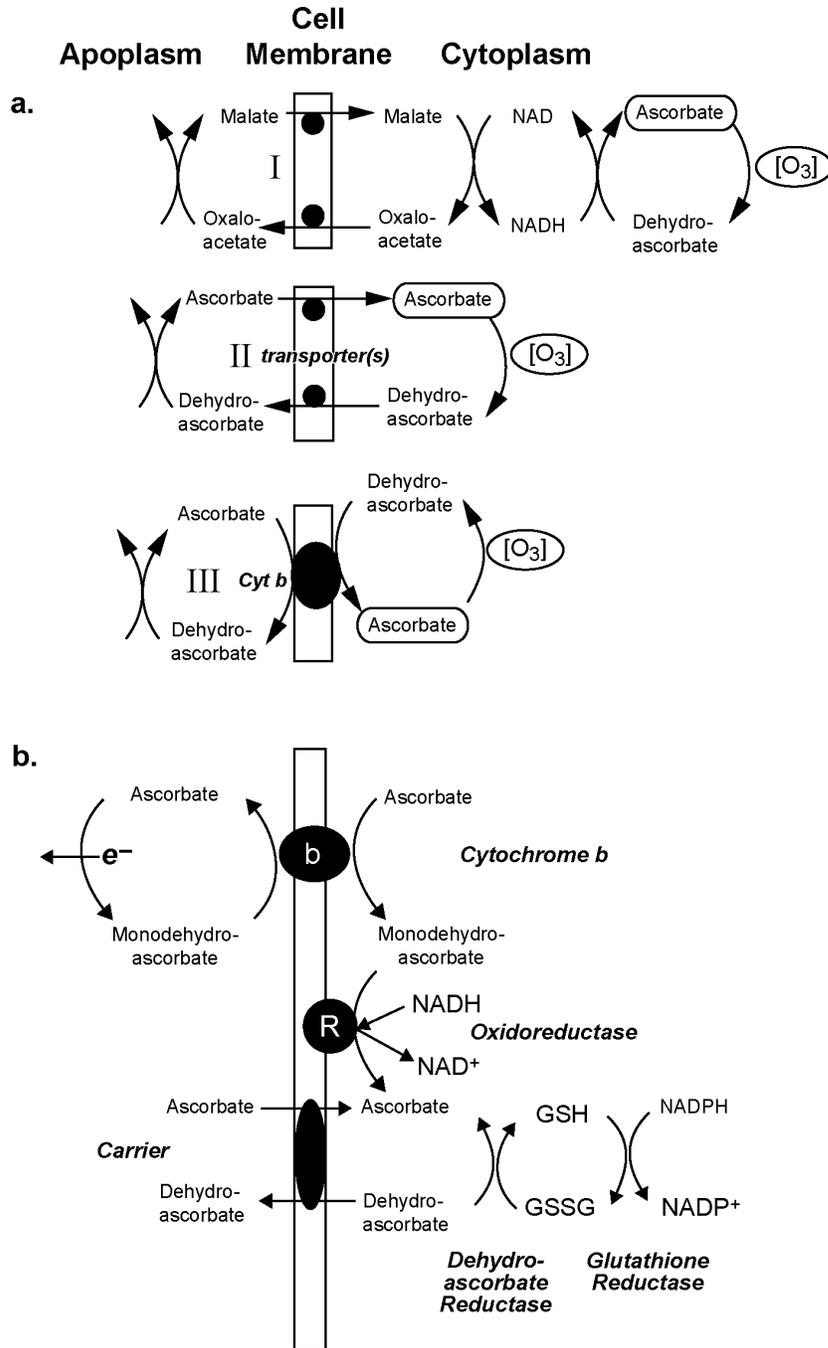


Figure AX9-12. The reaction of ascorbate within the apoplasm of the cell wall and its ultimate reduction/oxidations.

(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]).

1 as a cofactor rather than its antioxidant properties. In addition to its lowered antioxidant
2 capacity, the ascorbate-deficient *Arabidopsis* mutant *vtc1* (Conklin et al., 1996; Conklin and
3 Barth, 2004) may show suppressed growth due to lower mannose levels that are necessary for
4 cell wall formation. Ozone thus may suppress growth in these mutants through interference with
5 cell wall biosynthesis as well as through lower antioxidant protection.

6 The ascorbate peroxidase (APX, which uses ascorbate to detoxify peroxides) family
7 consists of at least five different isoforms, with isozymes in the apoplastic and cytosolic space.
8 Furthermore, most forms of ascorbate can move through the plasma membrane (Bichele et al.,
9 2000), making the levels of all forms of ascorbate interdependent and able to at least partially
10 influence each other. Dehydroascorbate (DHA) can be broken down into other smaller
11 fragments easily in vivo and represents a continuous loss of ascorbate from varied parts of the
12 cell if ascorbate is allowed to remain in the oxidized form in some regions. In fact, the turnover
13 rate in leaves is estimated to be from 2 to 13% per hour, depending upon species and
14 developmental age (Smirnoff et al., 2001). There are apparently three pathways for ascorbate
15 turnover (Figure AX9-12a). The typical reaction is a reduction of DHA into ascorbate from
16 which an oxidative step generates DHA. Pathway I requires a reductive step using NADH
17 external to the plasma membrane generated from internal malate using a malate/oxaloacetate
18 transporter. Pathway II uses a direct transporter of ascorbate/DHA. Pathway III moves the
19 required electron(s) through a cytochrome b system, maintaining two separate pools of
20 ascorbate/DHA (within the cytoplasm and within the wall). Each of the pathways (Dietz, 1997)
21 represented by Roman Numerals in the Figure AX9-12, require only one NAD(P)H molecule to
22 reduce the DHA molecule back to ascorbate. However, the transport properties and redox
23 potential of the cell differ for each pathway. The efficiency of the reduction of DHA is
24 dependent upon the redox coupling and the region in which the chemical species is located.

25 Turcsányi et al. (2000) exposed broad bean (*Vicia faba*) grown under two regimes in
26 duplicate controlled chambers: charcoal/Purafil filtered air (CFA) or (CFA) plus 0.075 ppm O₃
27 for 7 h/day for 28 days (chronic exposure) or exposed to 0.150 ppm for 8 h (acute exposure).
28 Responses of the two sets of plants were similar except for stomatal conductance, which was
29 50% lower in the chronically exposed plants. Plants grown under acute exposures developed
30 visible injury, while plants grown under chronic conditions developed no visible injury. Within
31 an hour of the start of the acute exposure, the stomatal conductance was reduced by nearly 40%

1 and assimilation was reduced by nearly 18% in the clean air plants; the reduction in conductance
2 was only 21% and assimilation 16% in the plants subjected to chronic O₃ exposures. The
3 assimilation was affected similarly in both cases, while the conductance showed less of a
4 percentage drop in the chronic O₃-exposed plants and began at a lower O₃ level. The similarity
5 of the assimilation indicated that the stomata were not limiting assimilation in either case before
6 acute exposure. More to the point, the decline in ascorbate in the apoplastic space due to the O₃
7 exposure was "...more often than not, on the borderlines of statistical significance." However, a
8 30% decline in ascorbate after 4 h of acute O₃ exposure in both cases was observed. This lack of
9 significance may be due to a relatively large standard error of the data, which in turn may be due
10 to the difficulty of extracting and measuring ascorbate from the apoplastic space in quantitative
11 terms.

12 The chemical reaction⁷ of ascorbate and O₃ is given by the molecular rate constant of
13 $4.8 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. This is some 50,000× the rate constant for ET. Of course, it depends upon the
14 relative concentration of ascorbate and ET, but it is likely that ascorbate is in higher
15 concentration than ET. One would then expect that the rate reaction of ascorbate with O₃ would
16 greatly dominate any possible reaction of O₃ with ET. For a concentration of ascorbate in the
17 range of 1 mM and for an O₃ concentration of about 0.1 ppm or $4.2 \times 10^{-9} \text{ M}$, the detoxification
18 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)
19 calculated an O₃ flux of about $1.6 \times 10^{-9} \text{ moles m}^{-2} \text{ sec}^{-1}$. With a wall thickness of $0.12 \times 10^{-6} \text{ m}$
20 and all the O₃ flux going into the wall region, this would give about $1.3 \times 10^{-2} \text{ mol m}^{-3} \text{ s}^{-1}$ or
21 $1.3 \times 10^{-5} \text{ M s}^{-1}$ flux, which is less than 10% the detoxification rate.

22 23 ***Glutathione***

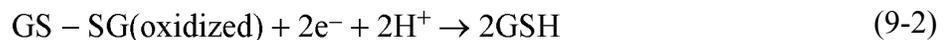
24 Many of the initial studies of O₃ exposure used high concentrations and measured only the
25 total sulfhydryl contents of the tissues. For example, in some of the earlier work, exposures of
26 tobacco to 1 ppm O₃ for 30 min induced a 15% loss of the total sulfhydryls (0.74 μmole/g-FW)
27 (Tomlinson and Rich, 1968). These results are similar to other studies at high O₃ levels (Dugger
28 and Ting, 1970). It is now suspected that the severe injury in their studies resulted in a massive
29 collapse of the cells and release of most of their internal constituents. Much of the oxidation

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.

1 thus observed may have been the result of chemical oxidations of the O₃ that subsequently
2 entered the damaged tissue. Even under milder conditions, changes in sulfhydryl components
3 have still been noted and any sulfhydryl on the surface of the cell would be at risk due to its high
4 reactivity with O₃ (Mudd et al., 1997b; Mudd et al., 1969). For example, the level of sulfhydryl
5 compounds within the protein of isolated chloroplasts declined about 66% when the chloroplasts
6 were subjected to O₃ (about 1 μmole O₃) exposure (Mudd et al., 1971).

7 At this stage, it is important to note that there are inherent problems with metabolic studies
8 of full tissues. The first is that most organs have several different types of tissues. For example,
9 leaves have, at the minimum, epidermal and vascular tissues and two types of mesophyll cells.
10 Each type of cell may be metabolizing quite differently and producing very different levels of
11 metabolites and enzymes. Furthermore, most pathways are well regulated and after any small
12 disruption, the pathway tends to return to near its former stability. Changes in the level of
13 enzymes are likewise difficult to measure. Many enzymes function below their maximum
14 activities. Their speeds of reactions are often increased through regulation, rather than through
15 the production of more enzyme.

16 Glutathione is a three-amino acid peptide, which has antioxidant properties due to its free
17 reducing sulfhydryl group (G-SH). Glutathione is generally kept in its reduced form by
18 glutathione reductase (GR) with the reaction:



20
21 GR has six isoforms⁸ within the chloroplast and six isoforms outside. The optimum activity
22 occurs at pH 7.8, suggesting it is located within the stroma of the chloroplast or in the cytoplasm
23 rather than in the cell wall, which is at pH 4-5 (Madamanchi et al., 1992). Clearly, an increased

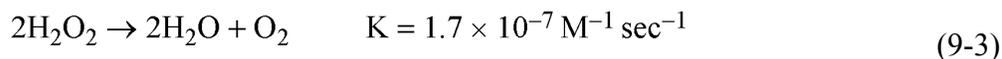
⁸An isoform is the same enzyme, with the same structure and perhaps within the same organelle, but its promoter region has different DNA codes. Thus, each protein segment is induced by different signals, and so its enzyme can be formed in response to different environments. This is in contrast to isozymes,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

1 expression of GR (generated through transgenic implants) is important within the chloroplast to
2 prevent some oxidations⁹ (Aono et al., 1995).

4 ***Catalase***

5 Catalase, even though it breaks down H₂O₂, does not appear to protect plants from O₃
6 exposures. Two principal reasons may cause this lack of reactivity: (1) catalase has a high K_m
7 for H₂O₂ and a low rate coefficient, and (2) catalase seems not to occur within the cell wall
8 regions but rather in the cytoplasm and peroxisomes (Buchanan et al., 2000). While a few
9 reports suggest that catalase is increased by exposure to O₃ (Azevedo et al., 1998), Booker et al.
10 (1997) found no effect of catalase activity in soybean until late in the growing season, and others
11 have found decreased catalase activity in wheat in response to O₃ (McKee et al., 1997).

12 Unfortunately H₂O₂ induced by some forms of wounding in mesophyll cells can lead to
13 induction of an increase in GSH and the transient production of catalase (Vanacker et al., 2000).
14 In general, it seems that catalase is not really involved primarily in the defense of the cell due to
15 O₃ attack but rather may be a secondary response. The reaction of catalase (Scandalios, 1993) is
16 as follows:



21 ***Superoxide Dismutase***

22 The varied compounds that O₃ can produce upon entering an aqueous solution are very
23 similar to those involved in the HR when plants are infected by an avirulent pathogen
24 (Figure AX9-10). The sequence of the plant response to the pathogen is (1) recognition of the
25 gene products of the pathogen by the plant (elicitor), (2) generation of an immediate
26 phytoresponse to attempt to localize the attack and its products, and (3) generation of a systemic
27 acquired resistance (SAR) to subsequent attack by the pathogen. Inducible defense responses are
28 phytoalexin synthesis and production of pathogenesis-related proteins (PR). One aspect of this
29 total response is the production of O₂⁻ and H₂O₂ by the cell (Lamb and Dixon, 1997). The

⁹Typically 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.

1 elicitor can generate a transient alkalinization of the apoplast, up to pH 7.2, caused by a lowering
 2 of the H⁺-pump rate and an increase in the H⁺-influx/K⁺-efflux exchange. Other effects include a
 3 weak accumulation of transcripts for PAL (phenylalanine lyase); a larger and rapid induction of
 4 glutathione S-transferase, GSH-P_x; oxidative cross linking of cell wall proteins which is blocked
 5 by ascorbate acid; generation of localized apoptosis; and rapid influx of Ca²⁺, which activates
 6 apoptosis among other pathways (Lamb and Dixon, 1997). These effects seem to be very similar
 7 to those induced by O₃ exposure (Sandermann, 1996, 1998).

8 The putative antioxidant enzyme SOD (Equation 9-4 and Table AX9-8) catalyzes the
 9 oxidoreductase reaction, which eliminates SO₂⁻ by dismutation (Bowler et al., 1992):

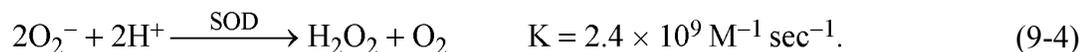


Table AX9-8. Superoxide Dismutase Isozymes and Isoforms

Reaction: 2H⁺ + 2 O₂⁻ → H₂O₂ + O₂			
Isozymes	M.W.	Isoforms	Cytolocation
Cu-Zn	20 kDa	<i>csd1</i>	
		<i>csd2</i>	Plastid
		<i>csd3</i>	Peroxisomal
Fe	23 kDa	<i>fsd1</i>	Mitochondrial
		<i>sd2</i>	
		<i>fsd3</i>	Plastid
Mn	23 kDa	<i>msd1</i>	Mitochondrial

1 The number, as well as the activity, of isozymes of each type of SOD in Table AX9-3 can
 2 vary with plant species. However, the isozymes that have been tabulated are Cu-Zn SODs, in
 3 cytosol and chloroplast; Fe-SOD, active in chloroplast stroma; and Mn-SOD, active in
 4 mitochondrial matrix (Karpinski et al., 1993). In the experiment demonstrating the activation of
 5 varied SODs, there were three Cu-Zn SODs (*csd1*, *csd2*, *csd3*), three Fe-SODs (*fsd1*, *fsd2*, *fsd3*),

1 and one Mn-SOD (*msd1*) (Kliebenstein et al., 1998). Ozone sensitivity was determined by
2 exposure of plants to 8 h of 0.33 ppm of O₃. *csd1* induced by O₃ and UV-B was one of the
3 earliest SOD increases and most pronounced responses for mRNA and protein. Also, some
4 increase in *csd3* (thought to be peroxisomal) was induced when the plants were exposed to a
5 high-intensity light pulse; *msd1* was unresponsive to the environmental stressors used here,
6 including O₃; and *csd2* (thought to be chloroplast) showed little increase. The *fsd1* isozyme
7 (present in the apoplasm) showed a slight decrease. On the other hand, an early report on snap
8 beans in which the experimenters used EDU, N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N-phenylurea
9 (Carnahan et al., 1978; Kostka-Rick and Manning, 1993), to prevent visible injury by O₃, 4-h O₃
10 exposure at 0.45 ppm was correlated with an increase in general enzyme activity of SOD, i.e.,
11 the level rose nearly 2.5× in 2 weeks at a level of 50 mg EDU per pot (Lee and Bennett, 1982).
12 It is believed that EDU may induce SOD, which then protects the plant. While gross assays of
13 enzyme activity have not proven to be very useful in understanding the mechanism of O₃ action,
14 in a well-crafted, long-term study involving ponderosa pine clones. Benes et al. (1995) stated
15 that “changes in antioxidant enzyme activity were not a consistent response to the O₃ fumigation,
16 but when observed, they occurred most often in the O₃-sensitive clone and in symptomatic,
17 fumigated branches...total (intra- and extracellular) activities of the antioxidant enzymes did not
18 appear to be good indicators of O₃ tolerance....”

19 Ozone exposure (70 ppb for 7 h/day for 14 to 42 days of exposure)¹⁰ caused an increase in
20 POD and a decline in SOD with no change in APX. No GSH was detected, but the
21 concentration of (ASC + DHA) was at 20 to 25 nmol/g-FW of extracellular fluid, compared with
22 2.4 to 3.0 mmol/g-FW of cell fluid. Glutathione within the cell was only 100 to 170 nmol/g-FW
23 of cell fluid. While these results are what one might expect for POD, the decline in SOD and
24 lack of change in APX are not what would be expected if protection was provided by SOD and
25 ascorbate. Yet as noted, because the rate of SOD reaction is many times higher than the rate of
26 O₃ entry, there may be no pressure to increase the SOD level.

¹⁰At a level of 70 ppb, the concentration of O₃ in air was about 3.06×10^{-6} mol/m³, which with the conductance of 0.042 mol/m² s, gives a flux rate of O₃ of 1.27×10^{-8} mole/m² s. Converting the SOD rate of 23 units/g-FW into a SOD rate within the apoplasmic space of 6.9×10^{-3} mol/m² s, or about 500,000 times the entry rate of O₃.

1 Some protection against visible injury (induced by 59 ppb daily mean O₃ for 14 h/day for
2 7 days) was observed in genetically modified tobacco plants with excess chloroplast SOD¹¹ (2 to
3 4 times higher), but less protection was observed in plants that had an excess of mitochondrial
4 SOD (8× higher) (Van Camp et al., 1994). In all lines, the conductance of the leaves dropped
5 about 50%, compared with the unmodified plants. There was a correlation with age of leaf (less
6 injury in younger leaves) that corresponded to that found in spruce trees in which the amount of
7 SOD declined in relation to the longer that needles were held on the tree (Polle et al., 1989).
8 A slightly different study, however, found no O₃ protection with varied SOD within the needles
9 (Polle and Rennenberg, 1991). Interestingly, in maize, the synthesis of SOD (any form) was not
10 stimulated by O₃ exposure (at 0.50 or 0.75 ppm for 8 h, variable times thereafter) but was by
11 exposure to 90% O₂ (Matters and Scandalios, 1987). It may be that this high level of O₃ does not
12 affect the SOD, or perhaps it stimulates and degrades the enzyme simultaneously.

13 The conclusions to be drawn from these results are not obvious. There seems to be SOD
14 (a Cu-Zn form) present in the apoplastic space of some plants, but it does not necessarily rise
15 with O₃ exposure. Thus, either its concentration is sufficient to provide protection or it is not
16 needed. Over expression of any SOD in other organelles may play a role, especially in the
17 chloroplast (Cu-Zn or Fe forms); however, it may be playing a secondary role due to other
18 effects of O₃ that generate conditions in which light can overload the chloroplast and generate
19 detrimental circumstances, including the production of SO₂⁻. In addition, SOD is
20 developmentally expressed in varied concentrations, so that long-term exposure to O₃ may alter
21 each leaf's developmental age and, in turn, alter what level of SOD is observed. In any case,
22 SOD does not seem to be the primary antioxidant system to protect against O₃.

23 24 ***Changes to the Plasmalemma***

25 Reports of “peroxidation” generally occur within unicellular organisms subjected to very
26 high levels of O₃ (in *Chlorella* [Frederick and Heath, 1970] and in *Euglena* [Chevrier et al.,
27 1990]). Heath (1987) determined that by the time biochemical events were altered and MDA
28 was produced in *Chlorella*, little permeability remained in the cells and most metabolic
29 pathways were greatly disrupted by the subsequent loss of substrates. In fact, MDA production

¹¹The SOD enzymes were from *Nicotiana plumbaginifolia* with appropriate transit sequence for targeting the correct organelle and expressed under control of cauliflower mosaic virus 35S promoter.

1 was concurrent with a high O₃ uptake by the cell, indicating a complete opening of the cell and
2 associated with the concurrent inability to plate the cells on a glucose medium (indicative of cell
3 death). Heath (1987) reached the conclusion that no one had proven that lipid oxidation was in
4 any way a part of the initial reactions of O₃ with the cell, a conclusion confirmed by Mudd et al.
5 (1997a). An excellent review regarding the initial action induced by O₃ within a plant
6 (Kangasjärvi et al., 1994) should be consulted. There is little data to show that lipids are
7 attacked by O₃ in any living system that was not previously severely injured by O₃. Most of the
8 data suggesting lipid attack by O₃ has been demonstrated in plants subjected to O₃ concentrations
9 of 0.5 to 1.0 ppm for several hours, during which gross wilting of the plant tissues usually
10 occurs, suggesting extreme water loss. It is not surprising that lipid and protein injury are
11 observed under these conditions. While those reports were useful in the 1960s and 1970s, they
12 are not especially insightful now when ambient levels of O₃ are rarely above 0.2 ppm.

14 **AX9.2.4 Wounding and Pathogen Attack**

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

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

29 Mehdy (1994) described a model of how an elicitor produced by the pathogen attack
30 activates a G-protein, which opens the inward-flowing Ca²⁺ channel. The flow of Ca²⁺ into the
31 cytoplasm raises the internal level (at the μM level) and activates a protein kinase that increases

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

Probe	Relevant Properties of Encoded Protein	Reference
PR-1	Acidic, extracellular; function unknown most abundant PR protein in tobacco; >90% identical to PR-1b and PR-1c	(Payne et al., 1989)
PR-2	Acidic, extracellular b-1,3-glucanase, >90% identical to PR-N and PR-O	(Ward et al., 1991)
PR-3	Acidic, extracellular chitinase; also known as PR-O; >90% identical to PR-P	(Payne et al., 1990)
PR-4	Acidic, extracellular; unknown function; homologous to C-terminal domain of Win1 and Win2 of potato	(Friedrich et al., 1991)
PR-5	Acidic, extracellular; homologous to thaumatin and bifunctional amylase/proteinase inhibitor of maize; also known as PR-R or PR-S	(Payne et al., 1989)
PR-1 basic	Basic isoform of acidic PR-1	(Payne et al., 1989)
PR-O'	Acidic, extracellular b-1,3-glucanase; approximately 55% identical to PR-2 group	(Payne et al., 1990)
Basic, glucanase	Vacuolar; approximately 55% identical to PR-2 group and PR-O'	(Shinshi et al., 1988)
Basic chitinase	Vacuolar; approximately 65% identical to PR-3 group	(Shinshi et al., 1987)
Acidic peroxidase	Extracellular; lignin-forming	(Lagrimini and Rothstein, 1987)

1 the activity of the plasma membrane NAD(P)H oxidase and generates O_2^- . Superoxide
2 dismutase converts O_2^- into H_2O_2 . Both O_2^- and H_2O_2 are responsible for the active oxygen
3 species response, which is believed to be a defense mechanism to kill the pathogen. In this
4 normal defensive reaction, a subsequent system induces either localized lipid peroxidation per se
5 or a membrane lipase to produce jasmonic acid or inositol triphosphate, which act as secondary
6 messages to activate the defense gene products.

7 Booker et al. (2004) found that G-proteins might be involved in the perception of O_3 in the
8 extracellular region using *A. thaliana* G-protein null mutants. The activation of a passive inward
9 flow of Ca^{2+} , e.g., by an O_3 -induced response, would serve the same function as activation of the
10 G-protein. Once the level of cytoplasmic Ca^{2+} rises, all else follows. It is suspected that
11 exposure of plants to O_3 does just that, as Castillo and Heath (1990) demonstrated — the in vivo
12 fumigation of bean plants both inhibits the outward-directed ATP-requiring Ca^{2+} pump and

1 increases the passive permeability of Ca^{2+} . It was thought that the calcium transporter system
2 has a sensitive sulfhydryl group which, if oxidized, would alter normal Ca^{2+} movements.
3 In addition, Dominy and Heath (1985) observed that the K^+ -activated ATPase (believed to be
4 involved in K^+ transport) was inactivated by in vivo exposure to O_3 and that inactivation was
5 traced to a sensitivity sulfhydryl. Mudd et al. (1996) argued that several amino acids are very
6 sensitive to O_3 , including any with an exposed sulfhydryl. Thus, the O_3 -induced change in Ca^{2+}
7 permeability may be the trigger to most, if not all, the wounding responses. However, the
8 difficult problem of proving that the cytoplasmic Ca^{2+} change is the first event in O_3 injury
9 remains.

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

18 **AX9.2.4.1 Peroxidases**

19 Increases in cytosolic and apoplastic peroxidase activity in response to O_3 are often
20 observed, but the reasons and outcomes of these changes have yet to be fully explained.
21 Increased activity is frequently correlated with O_3 injury. Dass and Weaver (1972) observed that
22 increases in peroxidase after O_3 injury was similar to that observed for plant infection by a virus.
23 Tingey et al. (1975) observed a 35% decrease in peroxidase activity immediately following O_3
24 exposure; however, within 24 to 48 h, activity had increased significantly and was above control
25 level and remained there throughout the remainder of the study. Dijak and Ormrod (1982) also
26 observed increases in peroxidase activity when two O_3 -sensitive and two O_3 -resistant varieties of
27 garden peas (*Pisum sativum*) were exposed to O_3 . Peroxidase activity was not related to cultivar
28 sensitivity nor to visible injury. Unfortunately, there are many peroxidases (Birecka et al.,
29 1976); therefore, any general increase is not specific. In ET-treated leaves, peroxidase reaction
30 products were found between the plasma membrane and the cell wall, suggesting that ET itself
31 could induce peroxidase activity (Abeles et al., 1989a,b; Birecka et al., 1976).

1 At the same time, others examined peroxidase reactions in general and found two types of
2 peroxidases (designated as acidic or anionic and basic or cationic, EC 1.11.1.7, but also listed as
3 EC 1.14.18.1). Many types of peroxidases are located in diverse organelles, and each seems to
4 be activated by different conditions (e.g., pH for anionic and cation types and substrates such as
5 guaiacol, syringaldazine, and ascorbate). Peroxidases belong to at least two groups, which
6 catalyze two separate reactions: (1) the reaction of H₂O₂ with ascorbate to form DHA, discussed
7 earlier (Thom and Maretzki, 1985), which is regenerated by plasma membrane electron transport
8 using a dehydrogenase (Gross and Janse, 1977) now believed to be a malate/oxaloacetate shuttle
9 through the membrane coupled to a NAD(P)H-cytochrome-b-reductoxidase; and (2) the reaction
10 with coniferyl alcohol (from phenylalanine through phenylalanine ammonia lyase) to form lignin
11 within the wall. The anionic peroxidase thought to be involved with lignification is within the
12 cell wall (Buchanan et al., 2000; Taiz and Zeiger, 2002). Some basic peroxidases are maintained
13 within the cell, while some are external to the cell. After wounding (Gasper et al., 1985;
14 Lagrimini and Rothstein, 1987), some basic peroxidases can be activated by processes leading to
15 the synthesis of stress ET (Yang and Hoffman, 1984) and/or by excess Ca²⁺ (Gasper et al., 1985).
16 Elicitor treatment of plants change a series of peroxidases, some of which are similar to those
17 seen in O₃-induced changes (see Table AX9-9).

18 The formation of lignin is due to the phenylpropanoid metabolism (Buchanan et al., 2000).
19 Tyrosine and phenylalanine are converted to cinnamic and *p*-coumaric acid, which are, in turn,
20 converted to *p*-coumaryl, coniferyl, and sinapyl alcohols, and then into lignins. Hence, the
21 peroxidase activity is often measured by one of these substrates (Espelie et al., 1986; Gasper
22 et al., 1985). However, it is questionable whether apoplastic peroxidase activity is limiting for
23 lignification; laccases have a prominent role as well. Also, availability of monolignols is critical
24 for core lignin formation, and it is unclear whether levels of these metabolites change in
25 response to O₃. Studies by Booker and others (Booker et al., 1991, 1996; Booker, 2000; Booker
26 and Miller, 1998) indicated that O₃ did not increase core lignin concentrations in foliage of
27 loblolly pine, soybean, or cotton; although levels of phenolic polymers and cell wall-bound
28 phenolics were elevated in soybean. Increased phenolic polymers appear to be lignin in acid-
29 insoluble lignin assays and may well be responsible, along with polyphenol oxidase, for the
30 stippling injury observed in O₃-treated plants. Cell wall function implies the transport of
31 peroxidase molecules out of the cell and, most likely, the regulation of their activities within the

1 wall space. These extracellular peroxidases may be observed by vacuum infiltration of buffer
2 into leaf air spaces and subsequent centrifugation of the tissues to remove the buffer with the
3 apoplastic enzymes that wash out (Castillo and Greppin, 1986). However, exposure to O₃
4 induces important changes in the plant. For example, extracellular peroxidase activity in *Sedum*
5 *album* leaves increased nearly 3-fold over that in the control plants after 2-h exposure to 0.40
6 ppm O₃ (Castillo et al., 1984). This O₃-induced increase in extracellular peroxidase appears to
7 be under the control of Ca²⁺ (Castillo et al., 1984; Heath and Castillo, 1987). Initially, no effect
8 on the anionic activity as measured with syringaldazine (specific electron donor for lignifying
9 peroxidases) was observed, yet 21 h later, the anionic peroxidase activity was increased, whereas
10 the cationic (ascorbate measured) peroxidase activity was decreased, in O₃-treated plants. This
11 suggests an immediate response (ascorbate peroxidase activation) and a secondary response that
12 activates the lignifying peroxidase via gene activation.

13 The rapid response of cationic peroxidase after O₃ exposure may not result from de novo
14 protein synthesis but from the secretion and direct activation by Ca²⁺ ions of enzyme molecules
15 already present in the tissue. Cationic peroxidases might attack the peroxides and, in this
16 manner, act as a detoxifying agent with ascorbate as the substrate in the apoplasm. The effect of
17 Ca²⁺ upon peroxidase activity is stronger at low H₂O₂ concentrations (Penel, 1986). Thus, one
18 can imagine that, when the H₂O₂ concentration is low, this peroxidase activation would have a
19 greater in vivo importance. Furthermore, the secretion of cationic peroxidases into the free
20 spaces as a result of O₃ treatment is accompanied by a simultaneous release of at least one of its
21 natural substrates (ascorbic acid); this cationic peroxidase exhibits a much higher affinity toward
22 ascorbate (up to 6-fold) than the anionic isozyme (Castillo and Greppin, 1986).

23 24 **AX9.2.4.2 Jasmonic Acid and Salicylic Acid**

25 Jasmonic acid (JA) and salicylic acid (SA) are considered to be regulators of the plant
26 defense response (Figure AX9-13) (Buchanan et al., 2000). They tend to respond more slowly
27 than ET, causing widespread effects in the plant tissues. Both seem to be heavily involved in
28 responses of the plant to O₃, once again linking the pathogen/wounding defense to O₃-induced
29 injury; however, their roles are far from clear.

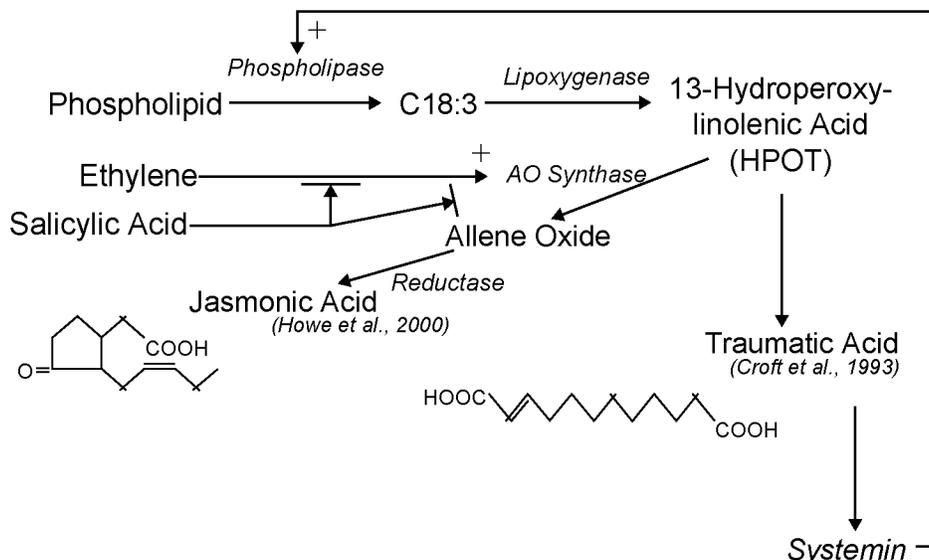


Figure AX9-13. The pathway leading from phospholipids to jasmonic and traumatic acid. The role of lipoxygenase and the production of a hydroperoxyl moiety from the unsaturated fatty acid is clearly demonstrated. More importantly, several of the enzymes within this pathway have been shown to be activated by oxidative conditions including O₃ exposure. The production of both of these acid species could lead to a general global response of a whole plant to the O₃ exposure of a single leaf.

Sources: Howe et al. (2000); Croft et al. (1993); Buchanan et al. (2000).

1 One of the lipoxidase isoforms is activated by pathogen infection (POTLX-3) within 6 h
 2 and accumulates for a week (Kolomiets et al., 2000). This enzyme is the first stage of the JA
 3 pathway which leads to 13-hydroperoxide linolenic acid (HPOT) which is converted either to
 4 allene oxide through AOS or to C6 aldehydes through hydroperoxide lyase. These aldehydes act
 5 as signaling agents via systemic (Sivasankar et al., 2000) or volatile odiferous compounds
 6 (oxylipins) that have been implicated as antimicrobial toxins (Froehlich et al., 2001).
 7 Interestingly, these compounds seem to target the chloroplast envelop where they interact with
 8 its metabolism. As HPOT and AOs are both implicated in plant defense and are activated by O₃,
 9 these interactions may be related to how chloroplast enzymes and their mRNAs are involved
 10 in O₃-induced injury.
 11

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

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

17 The working hypothesis is that O₃, which is not eliminated by antioxidants in the cell wall,
18 alters the properties of the plasma membrane. Specific polypeptides, indicative of these
19 antioxidants, are induced. If specific receptor molecules or channels on the membrane are
20 affected, the ionic balance within the cytoplasm is changed, leading to altered transcription or
21 translation of the genes controlling those and other types of polypeptides. Once this membrane
22 disruption occurs, the cell must mobilize repair systems to overcome the injury. Thus, carbon
23 and energy sources once destined for productivity, must be used in repair processes. Some of
24 these repairs are thought to result from the induction of specific genes. Photosynthesis is
25 inhibited by direct inhibition of some of the enzymes, through byproducts of O₃ attack or
26 by altered ionic balance. At the very least, the decrease in photosynthesis is a result of
27 an O₃-induced decrease in *rbcS* mRNA.

Table AX9-10. Proteins Altered by Ozone as Measured by Molecular Biological Techniques as mRNA Level or Other Gene Activity Rather than Enzyme Activity

Exposure	Physiological Events	Identified Proteins Fast Increase Response	Slow Increase Response	Decline	Examined, But No Change	Unknown Proteins	Reference
150/300 ppb for 6 h daily	Leaf curling; reduced growth	GST, PAL	Pxase, SOD		CAT, LOX1		Grosjean et al. (1994)
300 ppb for 6 h daily	10 bands of 10 RNA					AtOZI1 >> casein kinase II	Sharma and Davis (1995)
200/500/1000 ppb for 2 h	Wilting (8 h); premature senescence					3 plasma membrane proteins: 75-, 45-, 35-kDa peptides	Tokarska-Schlattner et al. (1997)
350 ppb for 1-6 h	Ethylene production; downward curvature; water logging	ACS-6			ACS-1, -2, -4, -5		Vahala et al. (1998)
300 ppb for 6 h	Necrosis in NahG and Cvi-0 (accumulating SA), not in Col-0	Chl SOD, cytAPX, GST1	Chl GPX	Cab mRNA, cyto SOD, chl GR			Rao and Davis (1999)
150 ppm for 6 h/8 and 14 days	Downward rolling of leaf; early senescence		BCB, ERD1, SAG21	Cab, <i>rbcS</i>	Atgsr2, MT1, SAG 12, SAG 13, SAG 19, SAG 20		Miller et al. (1999)
160 ppb for 3-72 h	Early senescence	GST1, VSP2	MT1		CCH		Mira et al. (2002)
(a) 250 ppb for 8 h; (b) 250 ppb for 2 h; (c) 175 ppb for 8 h/4 days	(a) little chlorosis or lesions; (c) growth retardation	GST Apx, CuZn-SOD	PAT1	Fe-SODI GR, cab, rbs			Conklin and Last (1995)

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

Exposure	Physiological Events	Identified Proteins Fast Increase Response	Slow Increase Response	Decline	Examined, But No Change	Unknown Proteins	Reference
200 ppb for 24 h		PR-1, PR-2a, PR-5, AtEDS1, AtGST1, AtGST2	PR-3b, PR-4		LOX2, AtOZI1, PAL, Lhcb, PAT1, HSP		Matsuyama et al. (2002)
250 ppb for 6 h	Lesion initiated on margin and spread inward					red1, on chromosome 1, single Mendelian trait	Overmyer et al. (2000)

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

GST = Glutathione synthase
 PAL = Phenylalanine ligase
 PR-1 = Promoter region 1
 Pxase = Peroxidase
 CAT = Catalase
 LOX1 = Lipoxygenase
 ACS-6 = ACC synthetase
 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

1 **AX9.2.5 Primary Assimilation by Photosynthesis**

2 **AX9.2.5.1 Photooxidation: Light Reactions**

3 Photooxidation refers to the oxidation of chlorophyll within the light reaction due to an
4 imbalance between light absorption and the CO₂ use to produce carbohydrates. It was
5 discovered in the 1920s and studied under the concept of chlorophyll bleaching and photo-
6 autooxidation (Asada, 1999; Rabinowitch, 1945). What generally occurs is that electron transfer
7 from H₂O to NADPH declines, and a light reaction overload occurs. The slowdown of electron
8 transfer may also be due to inhibition of the dark reactions, through the poor use of small
9 molecular weight carbohydrates or a lowered amount of the fundamental substrate CO₂.
10 To counteract these detrimental reactions, a series of “antioxidant” reactions exist, which
11 eliminate the buildup of oxidative intermediates.

12 A lowered CO₂ level, which can be caused by stomatal closure (Heath, 1996), blocks the
13 use of reduced plastoquinone (PQH₂) in Photosystem II through NADP reduction in
14 Photosystem I (Hankamer et al., 1997). The buildup of PQH₂ reduces the amount of Q_A,
15 resulting in a buildup of P₆₈₀⁺|Pheo⁻ species (the primary photoact). The inability to reduce this
16 radical leads to injury to the D₁ protein (32 kDa) and its fragmentation into 23-, 16-, and 10-kDa
17 fragments (Hankamer et al., 1997). Ozone exposure of bean plants leads directly to the loss of
18 this D₁ protein (Pino et al., 1995). The loss of D₁ stimulates the production of new D₁ (and its
19 mRNA). Also, the production of the oxidized form of P₆₈₀ (P₆₈₀⁺) is harmful to the plant, because
20 electron flow from water to P₆₈₀⁺ is limited, generating a P₆₈₀^T (the triplet form of P₆₈₀), which is
21 highly oxidizing and can lead to dangerous reactions. One form of protection is the use
22 of β-carotene to convert the triplet form back to its normal state; however, that reaction can lead
23 to the loss of β-carotene. Without the protection of β-carotene, oxygen reacts with oxidized
24 products to produce singlet state of O₂. This, in turn, can react with chlorophyll, leading to ring
25 breakage that, in essence, leads to chlorosis. These types of reactions do not seem to occur
26 often, but chlorosis is one form of visible injury, and loss of β-carotene has been reported.

27 Using a FACE system to expose soybean to elevated O₃, Morgan et al. (2004) found that,
28 in leaves at the top of the canopy, there was no effect on the maximum light-adapted apparent
29 quantum efficiency of PSII (φPSII,max), electron transport at growth [CO₂], and saturating
30 light (J_{sat}) nor on the probability of an absorbed photon reaching an open PSII reaction
31 center (F_v'/F_m); the quantum yield). There was a small, but significant, decrease in

1 photochemical quenching (qP) at the top of the canopy. As leaves aged, the decrease in qP
2 was significant as leaves began to senescence, likely due to losses of chlorophyll. The results
3 of these studies and others suggest that alterations to the dark reactions are much more common
4 than to light reactions (Farage et al., 1991; Farage and Long, 1999).

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

7 A large body of literature published since 1996 shows that O₃ exposure affects Rubisco
8 concentrations (Pell et al., 1997). Treatment of a variety of plants with O₃ at near-ambient levels
9 results in a loss of Rubisco and of the mRNA coding for both subunits of Rubisco (*rbcS*, small
10 and *rbcL*, large). In addition, oxidation of Rubisco by O₃-generated ROS may be an important
11 factor in suppressing photosynthesis (Pell et al., 1997). Increased carbonyl concentrations of
12 Rubisco are correlated with O₃ injury (Kanoun et al., 2002; Leitao et al., 2003). Because
13 Rubisco plays such an important role in the production of carbohydrates (Figure AX9-14), any
14 loss may have severe consequences for the plant's productivity.

15 Chronic O₃ exposure, both with and without elevated CO₂, significantly lowered
16 assimilation and leaf conductance of soybean in aging mature leaves (Fiscus et al., 1997; Reid
17 and Fiscus, 1998), which was associated with significant decreases in Rubisco content in aging
18 leaves. Noormets et al. (2001) also found that O₃ had the greatest effect on older leaves of aspen
19 clones using a FACE exposure facility in which areas of ambient CO₂ (daytime 360 ppm) and
20 ambient with added CO₂ (560 ppm), with added O₃ (97.8 ppb), and with added CO₂ and O₃, were
21 used. They found an O₃- induced decline in assimilation and in conductance, and subsequently
22 confirmed that the internal CO₂ (calculated for within the leaf) is not affected by O₃ exposure.
23 Higher levels of CO₂ increased the assimilation and lowered the conductance, maintaining the
24 internal to external CO₂ ratio identical to that found with the ambient CO₂ level, corresponding
25 to the theory of Farquhar et al. (1980). More to the point was that the stomatal limitation¹² was
26 not altered by O₃ exposure, with or without excess CO₂. It is critical to point out that mesophyll
27

¹²The limitation was defined as the ratio of stomatal resistance to the total resistance, which included the operating point of the assimilation (A) versus internal CO₂ concentration (C_i) curve and the resistance of the boundary layer. The operating point of the curve was defined as the internal CO₂ 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 ⁻²s of time and, thus, it is unclear whether the slope changes with added CO₂ and O₃.

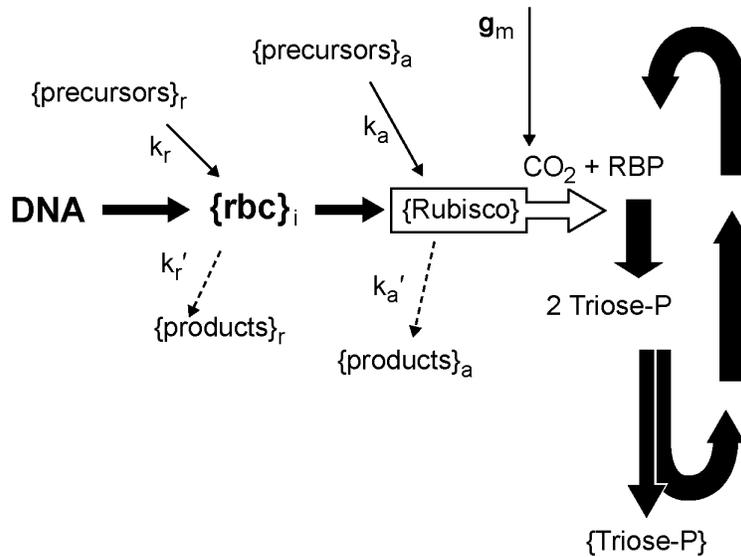


Figure AX9-14. The production of Rubisco and its Calvin Cycle pathway reactions. Two peptides are used to build Rubisco: *rbcS*, the small subunit produced by DNA within the nucleus; and *rbcL*, the large subunit produced by DNA within the chloroplast itself. Clearly both polypeptides must be closely 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 promotor region.

1 conductance is directly linked to internal CO_2 level¹³. So if C_i/C_o ($C_o = CO_2$ outside) is constant
 2 and g_s declines, then g_m must likewise decline. If, as it is argued, Rubisco levels are constant or
 3 at least increasing, then a regeneration of RuBP must be the cause of the decline in g_m . Farquhar
 4 et al. (1980) were more concerned with high levels of CO_2 and had little to say about O_3
 5 exposure.

6 In a similar study, Morgan et al. (2004) examined elevated O_3 using a FACE system to
 7 increase O_3 by 20% over the entire growing season. They examined O_3 effects from emergence
 8 through the entire life cycle to senescence. Leaf photosynthetic performance was measured
 9 using a LI-COR 6400 with integrated chlorophyll fluorescence capabilities to examine both dark
 10 and light reactions. This study found no effect of elevated O_3 on newly expanded leaves over the

¹³Respiration is generally small at saturating A and often is ignored. By transforming the term $\{A = g_s (C_o - C_i)\}$ into $\{A = g_s C_o - g_m C_o = (g_s - g_m) C_o\}$ where $g_m = g_s (C_i/C_o)$ or the mesophyll conductance in earlier literature.

1 growing season. There were little O₃-induced changes in the light reactions; however, as leaves
2 aged, there were significant changes in the dark reactions. For example, there were significant
3 losses in the maximal photosynthetic assimilation in saturating light (A_{sat}) and the maximum rate
4 of carboxylation (an in vivo measure of Rubisco efficiency), and maximum rate of electron
5 transport for the regeneration of RuBP (J_{max}). The findings showed the greatest impact of O₃ on
6 the oldest leaves and demonstrated the significant impact on seed production.

7 The level of carbohydrate within the cell has an effect upon the amount of mRNA for
8 Rubisco (*rbcS*). Experiments by Krapp et al. (1993) indicated that a decline in carbohydrate
9 levels is probably due to the increased production of control metabolites, such as fructose
10 2,6-bisphosphate, which can shut down important sugar production pathways. This report also
11 leads to a measure of half-time for the decline in *rbcS* of about 2 days¹⁴ when 50 mM glucose is
12 added to a cell suspension of *Chenopodium*. Also, the carbohydrate level was increased by cold
13 girdling the petioles of intact tobacco and potato plants. The levels of carbohydrate nearly
14 doubled in 5 days and the level of *rbcS* declined rapidly (reaching 25% after 12 h). A decline in
15 Rubisco followed, but more slowly (with an estimated half-time of about 108 h after a lag of at
16 least 12 hours). This, of course, is expected; the level of the enzyme would decline slowly with
17 a lag after a loss of the message.

18 A better estimation of the half-life of *rbcS* can be found in the Jiang et al. (1993) study of
19 the destabilization of the message by an antisense message. The wild-type *rbcS* in tobacco had a
20 half-life of about 5 h compared to that in the mutant with the antisense. It was argued that the
21 antisense message increased the degradation of the normal *rbcS*. The estimated half-life of *rbcS*
22 under O₃ fumigation is about 1 h (Pell et al., 1994). Although comparisons of these diverse
23 systems cannot be easily made, the normal half-life of *rbcS* may be closer to 5 to 10 h; and O₃
24 fumigation does not simply stop the transcription of DNA, but rather it alters the rate of
25 degradation, either independently of, or simultaneously with, transcription.

26 Williams et al. (1994) developed a correlation between the levels of ABA after water stress
27 in *Arabidopsis thaliana* leaves and the loss of *rbcS*. Although their data were not quantitative, the
28 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
29 about 2 to 4 h. Their work suggests that drought stress may alter the CO₂ metabolism by
30 changing enzyme relationships much more than by merely closing the stomata. If an ABA rise

¹⁴The amount of Rubisco drops from an initial 0.12 to a final amount of 0.04 μmole/g-FW s in 6 days.

1 is lowering *rbcS*, *rbcS* may not be a good marker of O₃ fumigation except under highly
2 controlled conditions.

4 **AX9.2.7 Carbohydrate Transformations and Translocation**

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

13 Many of the experiments with O₃ fumigation indicate that O₃ exposure decreases the net
14 growth or dry mass of the plant, but the mechanism is poorly understood. Generally the
15 decrease in assimilation is much less than the decrease in growth, but not always. Under many
16 conditions, the stomata will close partially, decreasing assimilation by a smaller factor. Only a
17 long exposure, or high levels of exposure for a short time, generate enough decline in Rubisco to
18 make the growth of the plant problematical. No convincing argument has linked the decrease in
19 growth with a small decline in assimilation, either by a conductance- or Rubisco-limitation.
20 Measures of assimilation with crops are frequently done on upper canopy leaves, which are the
21 last leaves to exhibit O₃ injury, while leaves deeper in the canopy exhibit injury and early
22 senescence. Crop root growth must be sensitive to these and other O₃ effects, because root
23 biomass is often suppressed early by elevated O₃.

24 Volin et al. (1998) found O₃ exposures statistically decreased leaf area ratio, specific leaf
25 area, leaf weight ratio, and root weight ratio in *Populus tremuloides* and two C3 grasses
26 (*Agropyron smithii* and *Koeleria cristata*) but not in *Quercus rubra* and in the C4 grasses
27 *Bouteloua curtipendula* and *Schizachyrium scoparium*. There was no statistically significant
28 change in any species in leaf conductance (4% level decline in *K. cristata*) nor in assimilation
29 (although there was a decline in assimilation at the 6% level for *P. tremuloides* and a decline at
30 the 1% level in *B. curtipendula*). They also reported a correlation between growth decline and
31 decreased stomatal conductance among all species.

1 Tradeoffs are made by plants. Birch grown in highly fertilized conditions exhibited a
2 greater leaf turnover when exposed to O₃, in that leaves not only formed faster but abscised
3 faster, presumably due to early senescence; whereas birch grown under poorer fertilized
4 conditions retained their leaves longer and had a greater respiration rate within those leaves
5 (Maurer and Matyssek, 1997). Again, one must be careful in comparing short-term versus long-
6 term exposures. Grulke et al. (2001) observed that maximum concentrations of carbohydrates in
7 1-year-old needles that had not abscised due to early senescence declined when subjected to
8 year-long exposures along an increasing pollution gradient. Furthermore, the monosaccharide
9 concentrations (along with starch) in fine roots were largely decreased, suggesting that needle
10 sugars were limiting, leading to root-sugar limitations. However, determination of the total
11 productivity and detailed balance of carbohydrate was impossible, because these were older,
12 larger trees and the data were taken over a full growing season. For a shorter-term exposure of 9
13 days, Smeulders et al. (1995) observed that O₃ appeared to increase the retention of labeled
14 photosynthates within the needle, and, at higher exposures (400 versus 200 or 0 mg/m³), the total
15 starch within the needle decreased, suggesting that less carbohydrate was produced within the
16 cell or perhaps that it was in compounds not measured.

17 Studies with Pima cotton (*Gossypium barbadense*), aspen (*Populus* spp.) and bean
18 seedlings (*Phaseolus vulgaris*) indicate that acute O₃ exposures inhibit export of the current
19 assimilate that provides carbohydrates to the roots from source leaves of cotton as well as recent
20 assimilate from the older leaves of aspen and bean (Grantz and Yang, 2000). Grantz and Yang
21 (2000) attempted to distinguish between potential mechanisms of O₃ phytotoxicity operating at
22 the level of the whole plant. Four hypotheses were tested by fumigating cotton: (1) O₃ exposure
23 reduces leaf pools of soluble sugars; (2) pruning leaf area and reducing source strength to match
24 that of O₃-treated plants reproduces O₃ effects; (3) pruning lower leaf area more closely
25 reproduces O₃ effects than pruning the upper leaf area; and (4) manipulating plant age and,
26 thereby, plant size to match O₃-treated plants reproduces O₃ effects. All were shown to be
27 incorrect. Under each of the above conditions, Grantz and Yang (2000) reduced the amount of
28 foliage to match that caused by O₃ injury. While the treatments reduced the biomass and leaf
29 area, they did not alter biomass allocation nor root function. They concluded that a simple loss
30 of foliage does not induce the changes in translocation to the roots to the same extent as does O₃
31 injury.

1 This finding by Grantz and Yang (2000) is important in that it suggests that O₃ can trigger
2 a plant-wide response that may be linked to alterations in signal transduction and the generation
3 of whole plant signals. Stitt (1996) suggested that "...allocation is regulated by long-distance
4 signals that act to influence growth of selected sinks and to modify the delivery of resources to
5 these sinks in parallel." Cooley and Manning (1987), citing McLaughlin and McConathy
6 (1983), suggested three possible ways that O₃ fumigation might alter translocation:
7 (1) malfunction of the phloem loading process, (2) increased translocation to leaf injury repair,
8 and (3) an altered balance between the leaf and sinks caused by reduced carbon fixation and a
9 greater demand for assimilate in the leaf.

10 Ethylene has been shown to reverse this sugar inhibition of development and to be
11 antagonistic to the ABA effect (Finkelstein and Gibson, 2002). However, these effects depend
12 greatly upon the developmental stage of the plant. Thus, the balance of the effectors (sugars,
13 ABA, and ET) may interact to generate the variation observed in the O₃-induced productivity
14 decline. For example, O₃ fumigation can induce a shift in the carbon transfer between roots and
15 shoot, and this shift can be amplified by mild drought (Gerant et al., 1996). Furthermore a
16 regulation of source-sink relations with the defense responses induced by elicitors was observed
17 by wounding the leaves of *Chenopodium rubrum* (Roitsch, 1999). Ethylene appears to be able to
18 repress the expression of extracellular invertase, which is critical for control and downloading of
19 sucrose derived from the translocational stream (Roitsch, 1999). In addition, the development of
20 *Arabidopsis* at high concentrations of glucose or sucrose is arrested by increasing the ABA level
21 (Coruzzi and Zhou, 2001).

22 Clearly more work is needed on the interactions between assimilation, translocation, and
23 source/sink relations with O₃ exposure. In these interactions, one must be aware of the
24 developmental age of the plants and their phytohormonal status.

25 26 **AX9.2.7.1 Lipid Synthesis**

27 Heath (1984) summarized several early reports of O₃-exposure induced lipid alterations.
28 Most concerned the production of MDA as a measure of lipid oxidation as well as the loss of
29 unsaturated fatty acids. However, a series of experiments by Sakaki and coworkers (Sakaki
30 et al., 1983, 1985) concentrated on one type of fumigation system and one metabolic pathway.

1 This literature provides the best, most complete story with regard to lipid metabolism and O₃
2 fumigation and suggests that O₃ injures cellular membrane systems via lipid destruction.

3 Sakaki and coworkers (Sakaki et al., 1983, 1985) used spinach, which is a sensitive plant
4 but which has not been much evaluated with respect to O₃ fumigation. While the O₃ level was
5 high (0.5 ppm), enough work has been done to be able to discern what is happening. The first
6 paper showed that chlorophyll bleaching does not begin until the plants have been exposed to O₃
7 for more than 10 h, whereas some MDA production begins with as little as 6 h exposure (Sakaki
8 et al., 1983). Consistent production of MDA, indicative of gross disruptions, occurred only after
9 8 h exposure (Sakaki et al., 1985), within the timescale when chlorophyll and carotenoid levels
10 begin to decline. Concurrently, the total fatty acid (FA) level decreased from ~481 to 358
11 nmol/cm² as the MDA level increased from 0.6 to 2.4 nmol/cm², indicating FA peroxidation
12 (Sakaki et al., 1985).

13 Sakaki et al. (1983) also studied the development of changes by cutting disks from exposed
14 leaves and floating them on water solutions for varied time periods (up to 24 h). This permitted
15 feeding experiments to be done easily, whereas the cutting gives rise to an additional wound
16 response and eliminates metabolite movement to and from other portions of the plant. The
17 floating experiments indicated that, after exposure, scavengers of singlet oxygen (¹O₂), such
18 as D₂O, and of hydroxyl radicals, such as benzoate and formate, have no effect on development
19 of the MDA response after 8 h of in vivo fumigation, while scavengers of (O₂⁻), such as tiron
20 and ascorbate, lowered the amount of MDA formed. By measuring metabolites immediately
21 after cessation of fumigation, they were able to show that ascorbate loss began with the onset of
22 fumigation, as did SOD loss. A lag time associated with the production of DHA suggested that
23 the reaction of ascorbate with fumigation did not immediately produce the oxidation product.
24 The first 4 h of exposure yielded 30 nmole/cm² of ascorbate loss with 5 nmole/cm² of DHA
25 production, whereas the second 4 h of exposure yielded 20 nmole/cm² of ascorbate loss with 20
26 nmole/cm² of DHA production.

27 Nouchi and Toyama (1988) exposed Japanese morning glory (*Ipomea nil*) and kidney bean
28 (*Phaseolus vulgaris*) to 0.15 ppm O₃ for 8 h. Under these conditions, little visible injury was
29 found with up to 4 h of exposure, while injury increased by ~50% after 8 h of exposure.
30 Morning glory produced more MDA than kidney bean, which produced the same as the zero-
31 time control. Morning glory also demonstrated a slight (5%) drop in MDGD

1 (monogalactosyldiacylcerol), with increases in PC (phosphatidylcholine), PG
2 (phosphatidylglycerol), PI (phosphatidylinositol), and PE (phosphatidylethanolamine) after 4 h.
3 Twenty-four hours later, the drop in MGDG (monogalactosyldiacylglycerol) was much larger and
4 was thought to be related to an inhibition of UDP-galactose galactotransferase due to a rise in
5 free fatty acids (FFAs) in the chloroplast. Note that the two distinct timescales involved in O₃
6 fumigation, immediately postfumigation and a day or so later, allows for comparison after the
7 plant metabolism responds to the fumigation event.

8 The pathway for the formation of MGDG and DGDG (digalactosyldiacylglycerol) is
9 located on the chloroplast envelope. Diacylglycerol (DG) arrives from either the endomembrane
10 system or the stroma and the enzyme UDP galactose-1,2-diacylglycerol galactosyltransferase
11 (UDGT) forms MGDG with galactose from UDP-galactose. Sakaki et al. (1990) suggested that
12 the O₃-induced inhibition of UDG T was due to a release of FFAs from within the chloroplast.
13 These FFAs are inhibitory to UDG T, but not to GGGT, which is stimulated by high
14 concentrations of Mg²⁺ (Sakaki et al., 1990). The Sakaki et al. (1990) data indicate that the in
15 vivo measured activities of both enzymes isolated after fumigation are not affected by O₃
16 fumigation. Both enzymes have sensitivity sulfhydryls, and both are located on the envelope.
17 Ozone, if it reaches those sulfhydryls, should inhibit these enzymes; yet inhibition was not seen.

18 It has been thought for years that tocopherols functioned as antioxidants in biological
19 systems (Tappel, 1972). Hausladen et al. (1990) examined the role of antioxidants in red spruce
20 (*Picea rubens* Sarg.) by following seasonal changes. They fit the level of tocopherol within the
21 needles (/g-FW) to the time of the year and found little change (fit as level = A + Bt + Ct²).
22 From this empirical fit, they found that the constant A was lower with higher levels of O₃. The
23 seasonal variation coefficients, B and C, were also lower, suggesting year-long low tocopherol
24 levels. Variation with the season is not particularly surprising, given that phytochrome action
25 may be linked to tocopherol biosynthesis (Lichtenthaler, 1977). Hausladen et al. (1990) reported
26 a significant (p < 0.05) trend in the difference between the high and low level of treatment;
27 although there was no discussion of why it occurred or what it meant in relation to metabolism.
28 Their major conclusion was that the antioxidant changes due to O₃ exposure may decrease cold
29 hardiness.

30 Sterols, believed to act as membrane stabilizers, have been investigated by several groups
31 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.

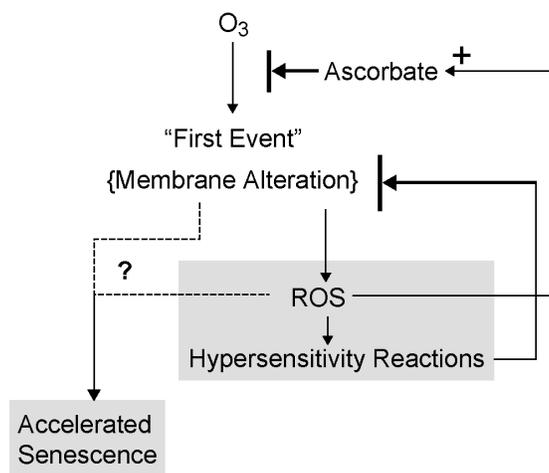


Figure AX9-15. Linkage of senescence with hypersensitivity reactions and the first event of O₃ attack of plants.

1 The understanding of how O₃ affects long-term growth and resistance to other biotic and
2 abiotic insults in long-lived trees is unclear. Often, the conditions to which a tree is subjected to
3 in one year will affect the response of that tree in the next year. This has been called “memory
4 effect”, although the term “carry-over” is preferred. In other words, a condition in an earlier
5 year sets the stage for a reaction in the next year; thereby giving a “cause-effect” scenario.

6 In perennial plant species, growth affected by a reduction in storage carbohydrates may
7 result in the limitation of growth the following year (carry-over effects) (Andersen et al., 1997).
8 Carry-over effects have been documented in the growth of tree seedlings (Hogsett et al., 1989;
9 Sasek et al., 1991; Temple et al., 1993; U.S. Environmental Protection Agency, 1996) and in
10 roots (Andersen et al., 1991; U.S. Environmental Protection Agency, 1996). Accumulation of
11 the carry-over effects over time will affect survival and reproduction. Data on the cumulative
12 effects of multiple years of O₃ exposures have been, for the most part, the result of 2- to 3-year
13 seedling studies. The difficulty of experimentally exposing large trees to O₃ has led to the tacit
14 assumption that seedling response to O₃ is a good predictor of large-tree response to O₃ (U.S.
15 Environmental Protection Agency, 1996).

16 The carry-over effects of O₃ exposures as observed in tree seedlings cited above by Hogsett
17 et al. (1989) have been termed “memory effects” by Langebartels et al. (1997) and proposed by
18 Schmieden and Wild (1995) to explain the sensitivity of spruce seedlings to frost in the winter
19 after having been exposed to O₃ during the previous summer. Norway spruce (*Picea abies* L.)
20 exposed to 80 ppb O₃ for a whole growing season, demonstrated visible injury symptoms the
21 following year when the new needle flush appeared (Langebartels et al., 1997). Additional
22 studies using Norway spruce and Scots pine (*Pinus sylvestris* L.) seedlings have shown similarly
23 delayed responses following O₃ exposures. Carry-over symptoms were noted to develop at
24 different times of the year, depending on the species of seedling exposed: in early spring for
25 Norway spruce, and in early autumn for Scots pine (Lange et al., 1989). Visible effects of O₃
26 exposures on spruce and pine may develop after a substantial delay during the “sensitive”
27 periods of the year when chlorophyll and needle loss normally occur. Norway spruce and Scots
28 pine differ in their sensitive periods because of the different needle classes normally remaining
29 on the tree (Langebartels et al., 1997).

30 Nutrient status of the tree during the overwintering phase of its life (Schmieden and Wild,
31 1995) and chronic exposure to ambient O₃ (less severe with fewer peaks of very high levels)

1 induce (1) mineral nutrient deficiency; (2) alterations of normal metabolism, including allocation
2 of carbohydrates and probably nitrogen; and (3) disturbance of normal transpiration and diurnal
3 cycling, leading to water stress. This condition, termed “Montane yellowing”, appears to be
4 related to nutrient deficiencies rather than senescence (although early loss of leaves and needles
5 occurs). While generalized low nutrient concentrations may not occur within the foliage,
6 localized deficiencies may. However, they are hard to observe or prove without a great deal of
7 work involving all portions of a tree and without a general hypothesis of what is occurring.
8

9 **AX9.2.9 Summary**

10 As the understanding of wounding responses of plants and more genome details and varied
11 plant mutants become available, the cellular and physiological responses of plants to O₃
12 exposures are slowly becoming clearer. However, more studies are needed on a larger variety of
13 species. Nevertheless, several key findings and conclusions can be highlighted:

- 14 (1) The entrance of O₃ into the leaf through the stomata remains the critical step in O₃
sensitivity. Not only does O₃ modify the opening of the stomata, usually closing it
partially, but O₃ also appears to alter the response of stomata to other stressful
situations, including a lowering of water potential and ABA responses. The
concentration of O₃ within the leaf is not the same as the external concentration due
to reactions within the leaf, but it is not “zero”.
- 15 (2) The initial reactions of O₃ within the leaf are still unclear, but the involvement of
H₂O₂ 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 O₃ (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 O₃ or product available to alter the plasma membrane
of the cells.
- 16 (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 Ca²⁺ is far from clear, but it would
seem that one of the primary triggers of O₃-induced cell responses is a change in
internal Ca²⁺ levels.
- 17 (4) The primary set of metabolic reactions that O₃ 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 Ca²⁺
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.

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

2 It is important to note that the dramatic strides in understanding the genetic makeup of
3 plants, gene control, and signal transduction/control over the last few years will likely accelerate
4 in the future. That understanding will translate into better models of the hypotheses listed above
5 and into more detailed schemes of how O₃ alters much of basic plant metabolism. Thus, while
6 understanding of how O₃ interacts with the plant at a cellular level has dramatically improved,
7 the translation of those mechanisms into how O₃ is involved with altered cell metabolism, with
8 whole plant productivity, and with other physiological facts remain to be more fully elucidated.

9
10

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40

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

2 **AX9.3.1 Introduction**

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

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

22 The impacts of these various factors on plant response to O₃ and other oxidants were
23 extensively reviewed in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996).
24 It was noted in that document that, since any combination of these factors may come into play at
25 some time in a plant's life history, "response will be dictated by the plant's present and past
26 environmental milieu, which also includes the temporal pattern of exposure and the plant's stage
27 of development." That document also stressed that both the impact of environmental factors on
28 response to oxidants and the corollary effects of oxidants on responses to environmental factors
29 have to be considered in determining the impact of oxidants on vegetation in the field. The
30 variability observed in plant responses to defined exposures to O₃, particularly under field

1 conditions, is a consequence of the influences of genetics and the range of environmental
2 variables.

3 In view of the large number of factors to be considered, this section focuses mainly on
4 situations in which there is clear evidence that environmental factors truly interact with oxidant
5 effects, i.e., they magnify or diminish the impact of O₃ and are *not merely additive* to it.
6 Conversely, it will cover situations where O₃ acts synergistically or antagonistically, but not
7 additively, with effects induced by other factors. It will also emphasize those interactions as a
8 result of which overall plant growth, development, and yield are adversely affected, rather than
9 the details of interactions at the mechanistic level, unless the latter are deemed to be essential to
10 an understanding of larger-scale effects.

11 Few studies reported since the 1996 document have systematically investigated
12 quantitative responses to O₃ exposure concurrent with other variables. Although the 1996
13 document cited almost 300 references pertaining to environmental interactions, and the present
14 review cites more than 350 new references, the bulk of the recently published work has
15 continued to be determined by the specific, and frequently narrowly focused, interests of
16 individual researchers or groups. Hence, the new findings are scattered and far from uniformly
17 distributed among the various subtopics. In some instances, little or no new research has been
18 published that adds to our understanding since the 1996 document. In such cases, the present
19 review is, therefore, restricted to summarizing the understanding that was current in 1996.

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

27 Although many reports have provided quantitative information on interactive effects,
28 in most cases the information simply describes a specific situation involving only two or three
29 levels of a variable. While this may be adequate to provide statistical information about the
30 existence of interactions with environmental factors, it does not permit the development of
31 response surfaces or models to show the form that any influence of such factors might take

1 on O₃ exposure-response relationships or how O₃ might quantitatively influence responses to the
2 factors in question. This, together with the fragmented information available on the effects of
3 most factors, has contributed to the relative lack of development of simulation models of
4 oxidant-environmental factor interactions. Yet, as noted by Taylor et al. (1994), the large
5 number of variables constrains the assessment of pollution effects by experimentation alone.
6 The only alternative is to use mathematical models to attempt to predict the outcome of
7 different O₃ and environmental factor scenarios, building up their complexity in stages.
8 The few models thus far used to investigate O₃ stress have been adapted from existing process
9 models of crop or tree growth that include limited numbers of physical or chemical variables
10 such as temperature, soil water stress, or nutrient deficiency. Taylor et al. (1994) provided a
11 listing of several simulation models developed for trees at the individual, stand, and regional
12 levels; these and many other models have been critically reviewed by Kickert and Krupa (1991)
13 and Kickert et al. (1999). However, regardless of whether such models are descriptive/empirical
14 or process/mechanistic, their outputs will always be associated with varying degrees of
15 uncertainty and require validation against observable responses wherever possible. Kickert et al.
16 (1999) also noted that very few of the models that have been described provide *risk assessments*
17 that address likelihood, in contrast to *consequence assessments* that address the magnitudes of
18 effects. Thus, even though capable simulation models of plant response to O₃ involving complex
19 mixes of many biological, physical, and chemical factors may be out of reach at the present time,
20 the use of newer mathematical approaches such as artificial neural networks (ANNs) has
21 enabled insightful analyses to be performed in several field studies involving numerous
22 micrometeorological and other environmental variables (e.g., Balls et al., 1996; Mills et al.,
23 2000).

24 Because the ensuing subsections deal with studies of O₃ interactions involving an
25 extremely wide array of biological, physical, and chemical factors in the plant's environment, it
26 is inevitable that many different exposure facilities and regimes have been used in these studies.
27 To provide specific information regarding the O₃ exposure concentrations, profiles, hours and
28 days of exposure (as well as the types of systems and facilities used for the exposures) would
29 add a wealth of detail that would do little to assist our understanding of the roles of environment
30 factors in modifying the impact of O₃ on vegetation or to facilitate our ability to estimate the
31 magnitudes of any such modifications. Thus, only experiments in which the exposure levels and

1 regimes were *within the bounds of ambient experience in North America* are discussed in the
2 ensuing subsections, regardless of the type of exposure profile used. The cutoffs used have
3 been ~200 ppb for peak hourly concentrations or for short-term exposures, ~100 ppb for daytime
4 means involving prolonged exposures for several hours, or a doubling of ambient levels in cases
5 in which enriched exposure levels were a function of ambient levels. Actual details of the
6 exposure regimes and conditions can, of course, be obtained from the original references but are
7 only stated here when any distinction is required between the effects of different exposure levels.
8 Hence, it should be understood that ensuing statements such as “. . .it was found that O₃
9 caused. . .” should always be read as “. . .it was found that exposures to O₃ [within the range
10 of those that have been measured in ambient air] caused.”
11

12 **AX9.3.2 Genetics**

13 The response of individual plants to O₃ is affected by several factors, including the
14 environment in which it is growing, competition with neighboring plants, ontogeny, and
15 genetics. This section examines the role of genetics in plant response to O₃. In addition, major
16 knowledge gaps in the understanding of genetic aspects of O₃ response are pointed out.

17 It is well known that species vary greatly in their responsiveness to O₃ (U.S. Environmental
18 Protection Agency, 1996). This again has been recently demonstrated for grassland species
19 (Bungener et al., 1999b; Bungener et al., 1999a; Franzaring et al., 2002; Nussbaum et al., 2000a;
20 Pleijel and Danielsson, 1997; Warwick and Taylor, 1995), wild herbaceous plants (Bergmann
21 et al., 1999; Danielsson et al., 1999), agricultural crops (Benton et al., 2000; Elagöz and
22 Manning, 2002; Fumagalli et al., 2001; Heagle and Stefanski, 2000; Köllner and Krause, 2003;
23 Nali et al., 2002; Nussbaum et al., 2000b; Ollerenshaw et al., 1999; Postiglione et al., 2000;
24 Renaud et al., 1997), horticultural shrubs and trees (Findley et al., 1997; Hormaza et al., 1996),
25 and forest trees (Bortier et al., 2000a; Guidi et al., 2001; Landolt et al., 2000; Matsumura, 2001;
26 Momen et al., 2002; Nali et al., 2002; Oksanen and Rousi, 2001; Pääkkönen et al., 1997; Pell
27 et al., 1999; Saitanis and Karandinos, 2001; Volin et al., 1998; Zhang et al., 2001). These
28 studies have shown a wide range of responses to O₃, from growth stimulation by a few species
29 such as *Festuca ovina* L. (Pleijel and Danielsson, 1997) and *Silene dioica* and *Chrysanthemum*
30 *leucanthemum* (Bungener et al., 1999b; Bungener et al., 1999a) to significant growth reduction,
31 depending on environmental conditions and exposure dose.

1 While determining the explanation for differences in species sensitivity to O₃ remains one
2 of the challenges facing plant biologists (Pell et al., 1999), a number of hypotheses have been
3 suggested. Reich (1987) proposed that variation in O₃ sensitivity could be explained by variation
4 in total uptake of the gas. Others have suggested that (1) fast-growing species are more sensitive
5 than slower-growing ones (Bortier et al., 2000b), (2) overall O₃ sensitivity may be closely linked
6 to root responses to O₃ (Warwick and Taylor, 1995), or (3) the relative ability of species to
7 detoxify O₃-generated reactive oxygen free radicals may determine O₃ sensitivity (Alscher et al.,
8 1997; Pell et al., 1999). Volin et al. (1998) suggested that the relative rate of stomatal
9 conductance and the photosynthesis rate at a given conductance both contribute strongly to
10 determining a species sensitivity to O₃. Likely, there is more than one mechanism determining
11 sensitivity, even in a single species.

12 Within a given species, individual genotypes or populations can also vary significantly
13 in O₃ sensitivity (U.S. Environmental Protection Agency, 1996). For example, the intraspecific
14 variation in O₃ sensitivity was a factor of two for *Phleum pratense* (Danielsson et al., 1999) and
15 *Trifolium repens* L. (Postiglione et al., 2000). A similar range of intraspecific variations in O₃
16 responses was demonstrated for clonal differences in *Betula pendula* by Pääkkönen et al. (1997)
17 and *Prunus serotina* (Lee et al., 2002). These examples of wide ranges within species responses
18 suffice to show that caution should be taken when ranking species categorically as having an
19 absolute degree of O₃ sensitivity (Davison and Barnes, 1998).

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

22 Plant response to O₃ is determined by genes that are directly related to oxidant stress and to
23 an unknown number of genes that are not specifically related to oxidants. The latter includes
24 genes that control leaf and cell wall thickness, stomatal conductance, and the internal
25 architecture of the air spaces. Although there is currently a great emphasis on individual
26 antioxidants that can be manipulated by molecular methods, the challenge is to determine the
27 relative contributions of all of the components to plant response and to understand the interplay
28 between them. Recent studies using molecular biological tools are beginning to increase the
29 understanding of O₃ toxicity and differences in O₃ sensitivity.

30 While much of the research in developing the understanding of O₃ responses has been
31 correlative in nature, recent studies with transgenic plants have begun to positively verify the

1 role of various genes and gene products in O₃ tolerance. The finding that the overexpression of
2 MnSOD in transgenic tobacco plant chloroplasts increased O₃ tolerance (Van Camp et al., 1994)
3 provided the first definitive proof of antioxidants key role in O₃ tolerance. Subsequently,
4 Broadbent et al. (1995) showed that the simultaneous overexpression of pea glutathione
5 reductase in both chloroplasts and mitochondria enhanced O₃ tolerance in transgenic tobacco.
6 Similarly, increased O₃ tolerance to O₃-induced foliar necrosis was shown for transgenic tobacco
7 plants overexpressing the cytosolic Cu/Zn-SOD gene (Pitcher and Zilinskas, 1996). Transgenic
8 tobacco plants expressing antisense RNA for cytosolic ascorbate peroxidase, which reduces
9 ascorbate peroxidase production, showed increased susceptibility to O₃ injury, suggesting a key
10 role in O₃ tolerance for the antioxidant ascorbate peroxidase (Örvar and Ellis, 1997).

11 The consensus among molecular studies of O₃ sensitivity is pointing to O₃ as triggering
12 salicylic acid, ethylene, and jasmonic acid production and that the signaling of these molecules
13 determines, in some cases, the O₃ susceptibility of plants (DeCaria et al., 2000; Moeder et al.,
14 2002; Nunn et al., 2002; Overmyer et al., 2000; Rao and Davis, 1999; Tamaoki et al., 2003;
15 Vahala et al., 2003a,b). Increased levels of jasmonic acid production in O₃-tolerant compared to
16 O₃-sensitive plants has been shown for *Arabidopsis* (Overmyer et al., 2000) and *Populus* (Koch
17 et al., 1998, 2000). Blockage of ethylene production by using antisense methods with
18 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase suggest strongly that
19 ethylene synthesis and perception are required for H₂O₂ production and cell death following O₃
20 exposure of *Lycopersicon esculentum* (Moeder et al., 2002). Ethylene signaling may have
21 multiple roles in O₃ tolerance determination as was demonstrated recently by Vahala et al.
22 (2003a,b) who found that, in *Populus tremula* × *P. tremuloides* hybrid clones differing in O₃
23 sensitivity, ethylene accelerated leaf senescence in sensitive plants under low O₃, but under acute
24 O₃, ethylene seemed to be required for protection from cell death.

25 While changing the expression of single antioxidant genes has proven very useful in
26 identifying possible mechanisms of O₃ sensitivity and tolerance (Kuzniak, 2002), it should be
27 noted that increased O₃ tolerance has not been shown in some studies of transgenic plants with
28 enhanced antioxidant production (Strohm et al., 1999; Strohm et al., 2002; Torsethaugen et al.,
29 1997). Clearly, ethylene production plays a role in O₃ sensitivity, but the roles of various
30 antioxidants in O₃ tolerance regulation are yet to be fully elucidated (Wellburn and Wellburn,
31 1996). It is unlikely that single genes are responsible for O₃ tolerance responses, except in rare

1 exceptions (Engle and Gabelman, 1966). Regulation of stomatal opening and leaf structure
2 (Elagöz and Manning, 2002; Fujita et al., 1992) are likely to play key roles in O₃ tolerance in
3 plants. Newly developing opportunities to examine simultaneous regulation of larger numbers
4 of genes are also likely to yield more clarification of the genes controlling O₃ tolerance (Desikan
5 et al., 2001; Matsuyama et al., 2002).

6 Attempts to demonstrate conclusive changes in antioxidant and protective pigments for O₃
7 sensitive and tolerant mature trees growing in the field have largely been unsuccessful (Tausz
8 et al., 1999a,b). However, evidence for antioxidant expression differences contributing to
9 differences in O₃ sensitivity of 4-year-old *Populus tremuloides* trees has been found (Wustman
10 et al., 2001).

11 12 **AX9.3.3 Environmental Biological Factors**

13 The biological factors within the plant's environment that may directly or indirectly
14 influence its response to O₃ in a positive or negative manner encompass insects and other animal
15 pests, diseases, weeds, and other competing plant species. Although such interactions are
16 ecological in nature, those involving individual pests, plant pathogens, or weeds, or agricultural
17 crop or forest tree species are considered in this section. More complex ecological interspecies
18 interactions are dealt with in Section AX9.5.

19 The different types of biological factors are dealt with separately, as in the 1996 O₃ AQCD
20 (U.S. Environmental Protection Agency, 1996). Still, it is important to recognize certain general
21 features of relationships of plants with the biological components in their environments:

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

27 Ozone and other photochemical oxidants may influence the severity of a disease or
28 infestation by a pest or weed, either by direct effects on the causal species, or indirectly by

1 affecting the host, or both. In addition, the interaction between O₃, a plant, and a pest, pathogen,
2 or weed may influence the response of the target host species to O₃. A perceptive overview of
3 the possible interactions of O₃-exposure with insect pests and fungal diseases has been provided
4 by Jones et al. (1994), based on a model system involving two insects and two pathogens
5 affecting cottonwood (*Populus deltoides*). Their study also included effects on the
6 decomposition of leaf litter.

7 In contrast to detrimental biological interactions, there are mutually beneficial relationships
8 or symbioses involving higher plants and bacteria or fungi. These include (1) the nitrogen-fixing
9 species *Rhizobium* and *Frankia* that nodulate the roots of legumes and alder and (2) the
10 mycorrhizae that infect the roots of many crop and tree species, all of which may be affected by
11 exposure of the host plants to O₃.

12 In addition to the interactions involving animal pests, O₃ may also have indirect effects on
13 higher herbivorous animals, e.g., livestock, due to O₃-induced changes in feed quality.

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

16 The 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) stressed the variability
17 in the reported effects of O₃ on host plant-insect interactions. Since relatively few plant-insect
18 systems have been studied, few consistent patterns of response have emerged, as noted in other
19 reviews such as those of Colls and Unsworth (1992), Heliövaara and Väisänen (1993), Whittaker
20 (1994), Docherty et al. (1997), and, most recently, Flückiger et al. (2002).

21 None of the studies reported in the past decade have clarified the situation in terms of
22 clearly consistent effects. A 1997 review by Docherty et al. (1997), for example, examined
23 17 reports of studies of aphid species on a range of hosts and classed the O₃ effects on aphid
24 performance as 35% positive, 41% negative, and 24% showing no significant effect.

25 A tabulation of 19 studies by Flückiger et al. (2002) gave the corresponding figures: 42%, 21%,
26 and 37%.

27 Other recent studies with the aphids *Schizolachnus pineti* and *Cinara pinea* on Scots pine
28 (*Pinus sylvestris*) and *Cinara pilicornis* on Norway spruce (*Picea abies*) have also yielded
29 variable results, but suggested that O₃ enhances aphid density on pine and aphid performance on
30 spruce (Holopainen et al., 1997; Kainulainen et al., 2000a). In an earlier study with
31 *Schizolachnus pineti* on Scots pine, Kainulainen et al. (1994) had observed no significant effects

1 of O₃-treatment on aphid performance. However, more recent observations of long-term effects
2 on aphid populations on aspen (*Populus tremuloides*) exposed to O₃ in a FACE system revealed
3 that O₃ significantly increased aphid populations while decreasing the populations of predatory
4 insects (Percy et al., 2002).

5 The observations of Brown et al. (1993) and Jackson (1995) led Whittaker (1994) and
6 Brown (1995) to suggest that aphid response was dependent on ambient temperature as well as
7 the dynamics of O₃ exposure and that growth tended to be stimulated with maximum
8 temperatures below ~20 °C but was reduced at higher temperatures. The present situation with
9 plant-aphid responses, therefore, remains confused and, although numerous suggestions have
10 been offered to explain specific findings, they are difficult to assemble into a coherent picture.

11 Variability has also been found with the interactions involving chewing insects. For
12 example, Lindroth et al. (1993) reported a small negative O₃ effect (8% reduction) on the growth
13 of gypsy moth larvae (*Limantra dispar*) on hybrid poplar (*Populus tristis* × *P. balsamifera*) but
14 no effect when growing on sugar maple (*Acer saccharum*). Ozone exposure reduced the growth
15 rate of the larvae of the bug *Lygus rugulipennis* on Scots pine, but enhanced the growth of larvae
16 of the sawfly *Gilpinia pallida* (Manninen et al., 2000). Costa et al. (2001) observed no
17 significant O₃ effects on the growth and fecundity of the Colorado potato beetle (*Leptinotarsa*
18 *decemlincata*) on potato (*Solanum tuberosum* L.) in greenhouse and field experiments.

19 Fortin et al. (1997), in a 2-year study of the forest tent caterpillar (*Malacosoma disstria*) on
20 sugar maple, observed that O₃ exposure increased the growth rate of female larvae in only one
21 year; fourth- and fifth-instar larvae also showed a feeding preference for treated foliage in that
22 year. However, studies based on open-air exposures of aspen indicated O₃-enhanced growth of
23 *M. disstria* in terms of pupal weight (Percy et al., 2002) and larval performance (Kopper and
24 Lindroth, 2003). Jackson et al. (2000) observed inconsistency in studies on the larva of the
25 tobacco hornworm (*Manduca sexta*) on tobacco (*Nicotiana tabacum*). In one year, feeding
26 on O₃-treated foliage resulted in significantly greater larval weight, whereas the increase was not
27 statistically significant in a second year although survival was increased. Also, oviposition by
28 hornworm moths was increased if ambient O₃ levels were increased by 70% and returned to
29 normal in ambient O₃ levels (Jackson et al., 1999).

30 Studies of the two-spotted spider mite (*Tetranychus urticae*) on white clover (*Trifolium*
31 *repens*) and peanut (*Arachis hypogaeae*) by Heagle et al. (1994) and Hummel et al. (1998)

1 showed that O₃-exposure stimulated mite populations on an O₃-sensitive clover clone and on
2 peanut. The lack of significant effects on mites on the O₃-resistant clover clone suggests that the
3 responses were host-mediated.

4 There, therefore, appears to be a clearer indication of the likelihood that increased chewing
5 insect and mite performance will result from O₃-induced changes in the host plant. However but
6 negative effects continue to be reported, indicating that the response is probably also being
7 determined, in part, by other environmental, genetic, or temporal variables.

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

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

26 The overall picture regarding possible O₃ effects on plant-insect relations, therefore,
27 continues to be far from clear. Only a few of the very large number of such interactions that
28 may affect crops, forest trees, and other natural vegetation have been studied. The trend
29 suggested in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) that O₃ may
30 enhance insect attack has received some support from a few recent studies. However, the

1 variability noted in most of the studies makes it clear that we are still far from being able to
2 predict the nature of any particular O₃-plant-insect interaction or its magnitude or severity.

4 **AX9.3.3.2 Oxidant-Plant-Pathogen Interactions**

5 Plant diseases are caused by pathogenic organisms, e.g., fungi, bacteria, mycoplasmas,
6 viruses, and nematodes. Ozone impacts on disease are briefly discussed in earlier reviews by
7 Ayres (1991) and Colls and Unsworth (1992) and, more recently, by Flückiger et al. (2002).
8 Biotic interactions with forest trees have been reviewed by Chappelka and Samuelson (1998);
9 Sandermann (1996) and Schraudner et al. (1996) have summarized molecular similarities and
10 interrelationships between necrotic O₃ injury to leaves and pathogen attack. A few recent
11 publications have added to our fragmented knowledge of O₃-plant-disease interactions and the
12 mechanisms involved, but there appear to have been no reports to date of studies involving
13 mycoplasmal diseases.

14 The 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) noted the concept put
15 forward by Dowding, (1988) “that pathogens and pests which can benefit from damaged host
16 cells and from disordered transport mechanisms are enhanced by pollution insult to their hosts,
17 whereas those pathogens and other symbionts which require a healthy mature host for successful
18 invasion are depressed by pollutant stress to their host.” The pathogens of the first type are
19 mostly facultative necrotrophic fungal parasites, whereas the second type are largely obligate
20 biotrophic fungi, bacteria, and viruses. Based on this distinction, the majority of the cases cited
21 in the 1996 document supported Dowding’s (1988) view, as have several more recent studies
22 summarized in Table AX9-11. However, there are also some contradictions.

23 Most investigations have focused on the incidence and development of disease on plants
24 previously or concurrently exposed to O₃, rather than on the corollary effect of disease on the
25 response to O₃. In all of the studies of facultative pathogens and the nematode studies, exposure
26 to O₃ tended to result in increased disease severity through increased spore germination or
27 increased fungal growth and development; although in the case of grey mold (*Botrytis cinerea*)
28 on kidney bean (*Phaseolus vulgaris*), this was only observed on an O₃-sensitive cultivar
29 inoculated with conidia (Tonneijck, 1994). After mycelial inoculation, O₃ exposure reduced
30 disease development in the O₃-sensitive cultivar, but no satisfactory explanation was offered to
31 account for the difference in response. With poplar leaf spot, *Marssonina tremulae*, on hybrid

Table AX9-11. Interactions Involving O₃ and Plant Pathogens

Host Plant	Pathogen	Effect of O ₃ on Disease	Effect of Disease on O ₃ Response	Reference
Obligate Biotrophs				
Bottle gourd (<i>Lagenaria siceraria</i>)	Powdery mildew (<i>Sphaerotheca fulginea</i>)	Increased in 50ppb O ₃ ; decreased in 100+ppb	Decreased; partial protection	Khan and Khan (1998a)
Cucumber (<i>Cucumis sativa</i>)	Powdery mildew (<i>Sphaerotheca fulginea</i> ;	Increased in 50ppb O ₃ ; decreased in 100+ppb	Synergistic increase in 50ppb O ₃ ; antagonistic decrease in 100+ppb; partial protection	Khan and Khan (1999)
Pea (<i>Pisum sativum</i>)	Powdery mildew (<i>Erysiphe polygoni</i>)	Decreased infection	Decreased; partial protection	Rusch and Laurence (1993)
Aspen (<i>Populus tremuloides</i>)	Leaf rust (<i>Melampsora medusae</i> f. sp. <i>tremuloidae</i>)	Increased severity	Not reported	Karnosky et al. (2002)
Hybrid poplar (<i>Populus trichocarpa</i> × <i>balsamifera</i>)	Leaf rust (<i>Melampsora larici-populina</i> or <i>M. allii-populina</i>)	Increased infection and severity	Increased sensitivity (synergistic)	Beare et al. (1999a)
Broad bean (<i>Vicia faba</i>)	Bean rust (<i>Uromyces viciae-fabae</i>)	Not reported	Decreased; partial protection	Lorenzini et al. (1994)
Facultative Necrotrophs				
Kidney bean (<i>Phaseolus vulgaris</i>)	Grey mold (<i>Botrytis cinerea</i>)	Increased from conidia on O ₃ -sensitive cultivar; decreased from mycelium	Not reported	Tonneijck (1994)
	Grey mold (<i>Botrytis cinerea</i>)	Increased infection	Not reported	Tonneijck and Leone (1993)
	White mold (<i>Sclerotinia sclerotiorum</i>)	Increased infection	Not reported	Tonneijck and Leone (1993)
Scots pine (<i>Pinus sylvestris</i>)	Annosus root and butt rot (<i>Heterobasidion annosum</i>)	Increased development*	Not reported	Bonello et al. (1993)

Table AX9-11 (cont'd). Interactions Involving O₃ and Plant Pathogens

Host Plant	Pathogen	Effect of O ₃ on Disease	Effect of Disease on O ₃ Response	Reference
Facultative Necrotrophs (cont'd)				
Loblolly pine (<i>Pinus taeda</i>)	Pitch canker (<i>Fusarium subglutinans</i>)	Increased development	Increased sensitivity	Carey and Kelley (1994)
Hybrid poplar (<i>Populus deltoides</i> × <i>nigra</i>)	Canker (<i>Septoria musiva</i> [= <i>Mycosphaerella populinum</i>])	Increased incidence	Not reported	Woodbury et al. (1994)
Hybrid poplar (<i>Populus trichocarpa</i> × <i>balsamifera</i>)	Leaf spot (<i>Marssonina tremulae</i>)	Increased spore germination and lesion growth after 100ppb O ₃ (30 days); decreased germination after 200ppb (15 days)	Not reported	Beare et al. (1999b)
Wheat (<i>Triticum aestivum</i>)	Blotch (<i>Septoria nodorum</i>)	Increased infection	Not reported	Tiedemann and Firsching (1993)
	Tan spot (<i>Pyrenophora tritici-repentis</i>)	Increased infection of disease-susceptible genotypes	Not reported	Sah et al. (1993)
Nematodes				
Tomato (<i>Lycopersicon esculentum</i>)	Root-knot nematode (<i>Meloidogyne incognita</i>)	Increased development	Increased foliar injury; reduced plant growth (synergistic)	Khan and Khan (1997); Khan and Khan (1998a)

* Increase completely countered by mycorrhizae (*Hebeloma crustuliniforme*).

1 poplar (*Populus trichocarpa* × *balsamifera*), low level exposures to O₃ increased disease (in
2 agreement with theory) but higher levels (200 ppb, 8 h per day for 15 days) reduced conidial
3 germination (Beare et al., 1999b).

4 The situation with obligate biotrophic pathogens is less consistent. The effects on powdery
5 mildew (*Sphaerotheca fulginea*) on both bottle gourd (*Lagenaria siceraria*) and cucumber
6 (*Cucumis sativa*) resembled the situation with the necrotrophic poplar leaf spot disease, since
7 low O₃ exposures increased disease severity (in disagreement with theory), although higher
8 levels decreased it. The decreased infection in the pea-powdery mildew (*Erysiphe polygoni*)
9 situation agrees with theory, but the situations with leaf rust (*Melampsora* sp.) on hybrid poplar
10 or aspen do not. However, these reports are in contrast to earlier reports included in the 1996 O₃
11 AQCD (U.S. Environmental Protection Agency, 1996) of observations with other species of
12 *Erysiphe* (Tiedemann et al., 1991) and *Melampsora* (Coleman et al., 1987). In contrast to the
13 recent report of a synergism with *Melampsora* on poplar, infections caused by the other
14 biotrophs (*Sphaerotheca*, *Erysiphe*, *Uromyces* spp.) reduced the severity of injury caused by O₃
15 (in agreement with numerous earlier reports), but only at high O₃ exposures in the case of
16 *Sphaerotheca* on cucumber. At low exposure levels, the disease and O₃ exposure acted
17 synergistically. The only other recent observations of such disease-related synergisms are the
18 tomato-nematode reports of Khan and Khan (Khan and Khan, 1997, 1998b).

19 It is, therefore, clear that the type and magnitude of exposure to O₃ plays an important role
20 in determining both the responses of both the disease organism and the host.

21 No recent studies involving interactions between O₃ and bacterial diseases appear to have
22 been reported since 1996. With regard to viruses, a laboratory study by Yalpani et al. (1994)
23 added to several reports of O₃ decreasing the severity of tobacco mosaic virus infection of
24 tobacco; and Jimenez et al. (2001) reported that previous O₃ exposure resulted in increased
25 adverse effects on tomato yield attributed to several viral diseases.

26 Similarities between the sensitivities of different cultivars or clones to O₃ and to specific
27 diseases have been noted. For example, Sah et al. (1993) found that the severity of injury caused
28 by tar spot and standard O₃ exposures of 12 wheat (*Triticum aestivum* L.) cultivars were closely
29 correlated (R² = 0.986). Such similarities appear to have a mechanistic basis, as several studies
30 have noted similarities in the molecular and biochemical changes that occur in plants infected
31 with pathogens and in O₃ exposed plants. Schraudner et al. (1992), Ernst et al. (1992), Eckey-

1 Kaltенbach et al. (1994a,b), Yalpani et al. (1994), and Bahl et al. (1995) have presented evidence
2 that O₃ exposures result in responses such as increased levels of salicylic acid, the signaling
3 agent for increased induced resistance to pathogens. This, in turn, leads to the activation of the
4 genes that encode defense proteins, including the so-called pathogenesis-related proteins. The
5 induction of such proteins might account for the decreased infection with *Sphaerotheca* and
6 *Melampsora* at higher O₃ exposures but does not account for increased infections seen at lower
7 exposure levels. The issue is discussed more fully by Sandermann (1996) and Schraudner et al.
8 (1996). More recently Sandermann (2000) has extended the theory relating O₃ exposure and
9 disease by suggesting that, because of O₃ “memory effects” in affected host plants that may
10 persist over weeks or months, analysis for various induced biomarkers of gene activation may
11 provide a useful tool for improving our ability to predict the outcome of O₃-plant-pathogen
12 interactions.

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

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

23 For obligate biotrophic fungi, bacteria, nematodes:

24 O₃ increased disease: 9 Increased susceptibility to O₃: 3

25 O₃ decreased disease: 15 Decreased susceptibility to O₃: 9

26 For facultative necrotrophic fungi:

27 O₃ increased disease: 25 Increased susceptibility to O₃: 2

28 O₃ decreased disease: 3 Decreased susceptibility to O₃: 4

29 Thus, although O₃ may reduce the severity, but not the incidence, of some of the diseases caused
30 by the obligate pathogens, the evidence overall indicates that with most diseases, their severity is
31 more likely to be increased by O₃ than not. However, the actual consequences will be specific to

1 the disease and level of exposure, and, most importantly, will be determined by environmental
2 suitability and epidemiological requirements for disease to develop. Conversely, some evidence
3 suggests that infection by obligate pathogens may confer some degree of “protection” against O₃,
4 a dubious benefit from the plant’s point of view.

6 **AX9.3.3.3 Oxidant-Plant-Symbiont Interactions**

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

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

26 In summary, the available evidence is far too fragmented and contradictory to permit
27 drawing any general conclusions about mycorrhizal impacts. The negative effects of O₃ on
28 mycorrhizae and their functioning that have been reported have not necessarily been found to
29 lead to deleterious effects on the growth of host plants. Thus, little has changed from 1991 when
30 Dighton and Jansen (1991) asked: “Atmospheric Pollutants and Ectomycorrhizae: More

1 Questions than Answers?”. Because of their important roles in ecosystems, mycorrhizae are
2 further discussed in Section AX9.6.

3 4 **AX9.3.3.4 Oxidant-Plant-Plant Interactions: Competition**

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

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

16 Although weak competition is the largest global cause of crop losses, little is known about
17 the impact of O₃ on crop-weed interactions. The topic does not appear to have been investigated
18 in recent years. We can only speculate as to the possible consequences of O₃ exposure on weed
19 competition based on our limited understanding of the effects on a few, mostly two-component
20 mixtures of cultivated species.

21 The tendency for O₃-exposure to shift the biomass of grass-legume mixtures in favor of
22 grass species, reported in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) has
23 been confirmed by recent studies. In a ryegrass (*Lolium perenne*) + clover (*Trifolium repens*)
24 mixture grown in an open-air fumigation system, clover growth was impaired by extended
25 exposures to above-ambient O₃, leaving patches for weed invasion (Wilbourn et al., 1995). An
26 open-top chamber study (OTC) by Nussbaum et al. (1995b) using the same species confirmed
27 the greater effect on clover but observed that the magnitude of the effect depended highly on the
28 pattern of O₃-exposures over extended growing periods. Low-level exposures shifted species
29 composition in favor of *Lolium*, but exposures to higher peak O₃ levels depressed total mixture
30 yield. With an alfalfa (*Medicago sativa*) + timothy (*Phleum pratense*) mixture, Johnson et al.
31 (1996a) noted that O₃ decreased alfalfa root growth and increased timothy shoot growth and

1 height. Nussbaum et al. (2000a) reported that, with increased exposure to O₃, well-watered red
2 clover (*Trifolium pratense*) plants suffered from increased competition from the grass *Trisetum*
3 *flavescens*, but the O₃ exposure also negatively affected grass growth, depressing overall total
4 yield. However, a greater adverse effect on *Trisetum* resulted from O₃-induced increased
5 competition when grown with brown knapweed (*Centaurea jacea*), a weed species.

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

11 Three studies have been reported on more complex plant associations. Ashmore and
12 Ainsworth (1995) studied mixed plantings of two grasses, *Agrostis capilaris* and *Festuca rubra*,
13 with two forbs¹⁵, *Trifolium repens* (a legume) and *Veronica chamaedrys*, exposed to O₃ in OTCs.
14 The proportion of forbs, *Trifolium* in particular, declined, especially when cut at biweekly
15 intervals. In a related study, Ashmore et al. (1995) used artificial mixtures of grasses and forbs
16 and transplanted swards of native calcareous grassland species and found that, regardless of
17 whether total biomass was adversely affected by exposures to O₃, higher exposures progressively
18 shifted species composition, usually at the expense of the forb species. The observed shifts in
19 competitive balance in favor of grasses is consistent with observations that many grass species
20 are less sensitive to O₃ than forbs. However, as previously shown by Evans and Ashmore
21 (1992), knowledge of the relative sensitivities to O₃ of the component species grown in isolation
22 or in monoculture does not always predict the impact of O₃ on the components in a mixed
23 culture.

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

¹⁵ Forb: any non-grassy herbaceous species on which animals feed.

1 concentrations resulted in decreased pine growth. They noted that this is contrary to the
2 frequently reported increased growth observed in reduced O₃ levels in the absence of
3 interspecific competition.

4 McDonald et al. (2002) classified four clones of aspen (*Populus tremuloides*) as either
5 competitively advantaged or disadvantaged, based on their height relative to the height of
6 neighboring trees, and exposed them to 1.5× ambient O₃ in a FACE facility over a 4-year period.
7 Competitively disadvantaged trees were proportionately more adversely affected by O₃ than
8 competitively advantaged or neutral trees (McDonald et al., 2002). However, one clone of the
9 disadvantaged trees demonstrated enhanced growth.

10 In summary, our present knowledge of how O₃ may affect the competitive interspecific
11 plant-plant relationships typifying the agricultural and natural worlds is very limited. However,
12 as noted in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996), “the
13 development and use of field exposure systems have permitted many recent studies of crop
14 species to be conducted at normal planting densities and hence have incorporated intraspecific
15 competition as an environmental factor.” Such facilities were used in most of the studies of
16 interspecific competition discussed above. But we are still far from being able to use small
17 model competing systems to extrapolate to the realities of natural ecosystem complexity.

18 19 **AX9.3.4 Physical Factors**

20 The physical features of a plant’s aerial and edaphic environments exercise numerous
21 controls over its growth and development. Thus, many of their effects may be modified by
22 exposure to atmospheric oxidants and, alternatively, plants may modify responses to such
23 exposures. As in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996), this
24 section focuses on the defining features of plant microclimate: light, temperature, relative
25 humidity (HR, or saturation vapor pressure deficit), and the presence and availability of water,
26 especially in the soil. Monteith and Elston (1993) suggested that light energy and mass of water
27 should be viewed as climatic resources and that the other two elements (temperature and
28 saturation vapor pressure deficit) be viewed as rate modifiers that determine how fast the
29 resources are used. The modifications of plant response by physical environmental factors has
30 recently been reviewed by Mills (2002).

1 Another physical feature of the microclimate, wind and air turbulence, which affects the
2 thicknesses of the boundary layers over leaves and canopies and, hence, affects gas exchange
3 rates (including the fluxes of O₃ and other oxidants into the leaves) is discussed elsewhere
4 (Section AX9.4).

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

9 10 **AX9.3.4.1 Light**

11 Plants are the primary producers of biomass on the planet through their ability to capture
12 light energy (by the process of photosynthesis) and convert it to the many forms of chemical
13 energy that sustain their own growth and that of secondary consumers and decomposers. Light
14 *intensity* is critical because the availability of light energy (a resource, *sensu* Monteith and Elston
15 (1993) governs the rate at which photosynthesis can occur, while light *duration* (i.e.,
16 photoperiod) profoundly effects development in many species. Although light *quality* (i.e., the
17 distribution of incident wavelengths) may also affect some physiological plant processes, there is
18 no evidence to indicate that such effects are of relevance to concerns over oxidant pollution,
19 except at the short wavelengths of UV-B. This topic is discussed in the context of climate
20 change in Section AX9.3.8.2, and as a stress factor per se affected by atmospheric O₃ in
21 Chapter 10. However, as noted above and in the 1996 O₃ AQCD (U.S. Environmental
22 Protection Agency, 1996), none of these features is controllable in natural field situations.
23 A brief discussion of light intensity-O₃ interactions is included in the review by Chappelka and
24 Samuelson (1998).

25 The conclusion in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) that
26 low light intensities and short photoperiods tended to increase susceptibility to foliar O₃-injury
27 may still be valid, but this may or may not translate into adverse effects on growth. For
28 example, Tjoelker et al. (1993) found that, when seedlings of sugar maple, a shade-tolerant
29 species, were grown in 7% full sunlight, O₃ reduced shoot and root growth, but had no
30 significant effect in 45% sunlight (a 6-fold increase). In contrast, the reverse was observed with

1 a shade-intolerant hybrid poplar, with the greater impact of O₃ occurring in the higher light
2 intensity treatment.

3 The greater sensitivity of maple in low light has also been confirmed in other studies.
4 Tjoelker et al. (1995) noted a greater O₃-induced inhibition of photosynthetic CO₂ assimilation in
5 shaded leaves than in leaves in full sunlight. However, in the absence of differences in stomatal
6 conductance, the effect was considered to be independent of O₃ flux; it appeared to be a
7 consequence of reduced chlorophyll contents and quantum efficiencies induced by O₃. In
8 contrast, Bäck et al. (1999), who also observed a greater inhibition of net photosynthesis by O₃ in
9 shaded leaves, reported decreased stomatal conductance. Although reduced conductance might
10 suggest reduced O₃ flux and, therefore, decreased adverse effects, the authors concluded that the
11 effects of reduced conductance were offset by long-term changes in leaf structure, leading to less
12 densely packed mesophyll cells and greater internal air space within the leaves. Morphological
13 differences between lower and upper crown leaves of black cherry (*Prunus serotina*) have been
14 suggested as the basis for the greater O₃-susceptibility of the lower crown leaves (Fredericksen
15 et al., 1995). Bäck et al. (1999) also observed accelerated foliar senescence induced by O₃ on
16 shaded leaves, a response also noted by Topa et al. (2001). Sensitivity to O₃ was found to be
17 increased in shade- but not sun-leaves of shade-tolerant red oak (*Quercus rubra*) (Samuelson and
18 Edwards, 1993). Similarly, Mortensen (1999) observed that seedlings of mountain birch (*Betula*
19 *pubescens*) grown in 50% shade suffered greater foliar injury from O₃ than those grown in full
20 sunlight.

21 Not all shade-intolerant species exhibit greater reductions in photosynthesis and growth
22 due to O₃ when grown in full sunlight. Higher than ambient levels of O₃ failed to inhibit
23 photosynthesis in leaves of shade-intolerant yellow poplar (*Liriodendron tulipifera*) grown in
24 nearly full sunlight (Tjoelker and Luxmoore, 1991). Greater foliar injury in the lower, shaded
25 leaves of shade-intolerant black cherry trees and saplings, was attributed to higher stomatal
26 conductance and greater O₃ uptake relative to net photosynthetic rate (Fredericksen et al.,
27 1996a). However, in a 3-year study of Norway spruce seedlings in OTCs, Wallin et al. (1992)
28 observed that photosynthetic efficiency was more adversely affected by O₃ in high than in
29 low light.

30 The suggestion of greater sensitivity to O₃ of shade-tolerant species in low-light conditions
31 and the greater sensitivity of shade-intolerant species in high light is somewhat of an

1 oversimplification when dealing with mature trees, for which light intensity varies considerably
2 within the canopy because of shading. Chappelka and Samuelson (1998) noted that the
3 interaction between sensitivity to O₃ and the light environment in forest trees is further
4 complicated by developmental stage, with seedlings, saplings, and mature trees frequently giving
5 different results. Topa et al. (2001) also cautioned that O₃ effects on leaf-level photosynthesis
6 may be poor predictors of the growth responses of sugar maple in different light environments.

7 In high-light intensities, many species exhibit some degree of photoinhibition of the
8 photosynthetic process through the overloading of the mechanisms that protect the
9 photosynthetic reaction centers in the chloroplasts. Guidi et al. (2000) reported complex
10 interactions between high-light intensities (inducing photoinhibition) and O₃ exposures in kidney
11 bean with high intensities tending to enhance the detrimental effect of O₃ on photosynthesis.
12 One of the studies in the extensive European Stress Physiology and Climate Experiment-wheat
13 (ESPACE-wheat) program (Bender et al., 1999), conducted in 1994 and 1995, included an
14 investigation of the effects of climatic variables on yield response to O₃ using two simulation
15 models, AFRCWHEAT2-O₃ and LINTULCC (Ewart et al., 1999; Van Oijen and Ewart, 1999).
16 Among the observed trends, it was noted that relative yield loss of wheat due to elevated O₃
17 tended to increase with light intensity. In contrast, Balls et al. (1996) used ANNS to investigate
18 microclimatic influences on injury caused by O₃ to clover (*Trifolium subterraneum*) and found
19 that, especially at mid-range cumulative O₃ exposures (350 to 500 ppb-h), injury tended to
20 decrease with increasing light intensity. Similar observations by Davison et al. (2003) of foliar
21 injury to wild populations of cutleaf cone flower (*Rudbeckia laciniata*) exhibiting a range of
22 PAR levels within their canopies led the authors to conclude that the variation in injury
23 symptoms observed was “unlikely to be due to differences in ozone flux and more likely to be
24 due to variation in light.” Antonielli et al. (1997) found evidence indicating that the high
25 sensitivity of the bioindicator tobacco cultivar *Nicotiana tabacum* cv. Bel-W3 is partly
26 determined by its high photosynthetic electron transport rates at high-light intensities, which
27 exceed the capabilities of the plant to dissipate energy and oxyradicals.

28 The 1996 O₃ AQCD referred to the important role of light in controlling stomatal opening
29 and suggested that light duration (i.e., photoperiod) might dictate the actual uptake of O₃ to some
30 degree. However, it should also be noted that Sild et al. (1999) found that clover plants could

1 suffer foliar injury even if they were exposed to O₃ during the dark period of the day-night cycle,
2 when stomatal conductance is at its lowest.

3 A possible indirect effect of light intensity was noted by Reiling and Davison (1992) in
4 their study of the O₃-tolerance of common plantain (*Plantago major* L.) plants grown from seeds
5 collected from populations at 28 different sites in Britain. Ozone-tolerance, defined in terms of
6 plant growth, was found to be a function of both previous O₃-exposure history and hours of
7 bright sunshine during the year before the seeds were collected. However, the authors cautioned
8 that, since tropospheric O₃-formation is itself dependent upon irradiation, the observation does
9 not necessarily imply a direct effect of light intensity on the plants' response to O₃.

10 The only recent studies concerning interactions with light quality appear to be those
11 involving O₃ and UV-B as a component of climate change. These are dealt with in Section
12 AX9.3.4.8.2. The effects of photoperiod on response to O₃ or the converse do not appear to have
13 received any recent attention.

14 Although the intensity, quality, and duration of light are not controllable in the natural
15 world, the interactions of O₃ with light intensity, in particular, clearly have relevance to the
16 growth of shade-tolerant and shade-intolerant species in mixed forest stands. It appears that the
17 nature of light intensity-O₃ interactions may depend upon the type of light environment to which
18 the species are best adapted, with increased light intensity increasing the sensitivity of light-
19 tolerant and decreasing the sensitivity of shade-tolerant species to O₃. Although there is
20 certainly some evidence to the contrary, this hypothesis is a reasonable summation of current
21 understanding with regard to O₃-light intensity interactions.

22 23 **AX9.3.4.2 Temperature**

24 “Temperature determines the start and finish and rate and duration of organ growth and
25 development” (Lawlor, 1998). Such processes depend on fundamental physiological activities
26 that are mostly enzyme-mediated and whose kinetics are directly affected by temperature. Since
27 the processes of enzyme deactivation and protein denaturation also increase as temperatures rise,
28 each enzymatic process has a unique optimum temperature range for maximal function.
29 However, the optima for different processes within the plant vary appreciably and, hence, the
30 optimum temperature range for overall plant growth is one within which all of the individual
31 reactions and vital processes are *collectively functioning optimally*, not necessarily *maximally*.

1 Furthermore, individual features of plant development (e.g., shoot and root growth, flowering,
2 pollen tube growth, fruit set, seed development) have different specific optima, so that
3 differential responses to temperature occur, leading to temperature-induced developmental
4 changes. For example, despite increased assimilation, increased temperatures may result in
5 decreased grain yields of crops such as wheat, because the growing season is effectively
6 shortened by a more rapid onset of senescence (Van Oijen and Ewart, 1999).

7 Rowland-Bamford (2000) noted that a plant's response to temperature changes will depend
8 upon whether it is growing at its near optimum temperature for growth or its near maximum
9 temperature and whether any increase in mean temperature results in temperatures rising above
10 the threshold for beneficial responses. Impairment by O₃ of any process may be thought of as
11 being analogous to a downward shift below and away from the temperature optimum or an
12 upward shift above and away from the optimum. Since a temperature rise toward the optimum
13 would result in a rate increase, the combined effects of O₃ and such an increase might neutralize
14 each other, while the effects of O₃ and a decrease in temperature would likely be additively
15 negative. Above the optimum temperature, the situations would be reversed with the effects of
16 increased temperatures and O₃ being additively negative, and decreasing temperatures
17 counteracting any negative effect of O₃. Thus, it is difficult to generalize about the interactions
18 of temperature and O₃ on overall plant responses such as growth in which the different
19 temperature-rate relationships of different growth components are merged, because they depend
20 upon the relationship of any temperature changes to the optimum for a species.

21 Studies of the effects of temperature on the impact of O₃ have increased recently because
22 of an increased need to understand the consequences of global warming as a component of
23 climate change. Direct interactions of temperature with O₃ are reviewed here, but the issues are
24 addressed again in Section AX9.3.8.1 in relation to changes in atmospheric CO₂ levels.

25 The 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) stressed the
26 interdependence of the temperature within the tissues of the leaf (where the various temperature-
27 sensitive processes occur) on three distinct components: the ambient air temperature, the heating
28 effect of incident infrared radiation during the photoperiod, and the evaporative cooling effect
29 caused by transpirational loss of water. It also cautioned that, especially in experiments using
30 controlled environment chambers, the effects of temperature could well be confounded with
31 those of humidity/vapor pressure deficit (VPD). Temperature and VPD are strongly interrelated,

1 and VPD plays an important role in regulating stomatal transpiration. Because of the role that
2 evaporative cooling plays in determining internal leaf temperatures, any factor that causes
3 stomatal closure and reduced conductance inevitably leads to increased leaf temperatures. Such
4 interactions add to the difficulties in distinguishing the effects of temperature from those of other
5 factors, as actual leaf temperatures are rarely measured and reported.

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

29 A study of black cherry seedlings and mature trees in Pennsylvania, using
30 micrometeorological variables aimed to predict O₃ uptake, found temperature to be unimportant
31 (Fredericksen et al., 1996b), but in the study of populations of common plantain referred to in

1 Section AX9.3.4.1, Reiling and Davison (1992) noted a weak, positive correlation between mean
2 temperature at the collection site and O₃ tolerance (based on growth rate) of the different
3 populations. In contrast, Danielsson et al. (1999) collected genotypes of *Phleum arvense* from a
4 wide range of Nordic locations and found a positive effect of temperature on the growth of
5 genotypes from locations with higher summer temperatures, but sensitivity to O₃ did not vary
6 systematically with geographic location.

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

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

28 The 1996 O₃ AQCD also noted that O₃ adversely affects cold hardiness of herbaceous
29 species. More recently, Foot et al. (1996, 1997) observed winter injury and decreased growth in
30 low-growing perennial heather *Calluna vulgaris* exposed to O₃ (70 ppb, 8 h/day, 5 days/week for
31 6 months) during the winter (6.8 °C mean), but found no significant effects from the same

1 exposures during the summer (12.3 °C mean). Although Potter et al. (1996) observed a similar
2 situation with the moss *Polytrichum commune*, the reverse was found with the moss *Sphagnum*
3 *recurvum*.

4 In summary, unequivocal evidence exists that O₃ causes sensitization to the adverse effects
5 of low temperatures, but there is no clear pattern in the evidence regarding the effects of
6 temperature on O₃ response. The many contradictory responses to temperature and O₃ probably
7 reflect our lack of detailed knowledge of the temperature optima for the different growth
8 components of the studied species. The topic of temperature-oxidant interactions is revisited
9 later in Section AX9.3.4.8 in the context of global warming as a feature of climate change.

11 **AX9.3.4.3 Humidity and Surface Wetness**

12 The moisture content of the ambient air (or its VPD) is a rate modifier (*sensu* Monteith and
13 Elston (1993)) and an environmental regulator of stomatal conductance. Both of the previous O₃
14 AQCDs (U.S. Environmental Protection Agency, 1986, 1996) concluded that the weight of
15 evidence indicated that high RH (=low VPD) tended to increase the adverse effects of O₃,
16 principally because the stomatal closure induced in most situations by O₃ is inhibited by high
17 RH, leading to increased O₃ flux into the leaves.

18 Recent reports have confirmed this role of RH. The studies by Balls et al. (1995, 1996)
19 and Ball et al. (1998) showed that VPD was an important determinant of O₃-induced injury
20 and reduced growth in two species of clover, *Trifolium repens* cv. Menna and *T. subterraneum*.
21 However, Mills et al. (2000) found it to be unimportant in the case of *T. repens* cv. Regal. Such
22 difference between cultivars is not unexpected, because considerable differences also occur
23 among species and genera. For example, Bungener et al. (1999a) studied 26 Swiss grassland
24 species and found clear evidence that O₃ injury increased with decreased VPD (i.e., increased
25 RH) in only eight species. However, the 1995 data from the European cooperative study of O₃
26 injury, which involved 28 sites in 15 countries and six crop species, led to the development of
27 two 5-day critical-level scenarios involving O₃-exposure (calculated as the AOT40 index) and
28 mean VPD (0930-1630h): 200 ppb-h at >1.5 kPa, and 500 ppb-h at < 0.6 kPa (Benton et al.,
29 2000).

30 With forest tree species, Fredericksen et al. (1996b) found significant correlations between
31 stomatal conductance of black cherry leaves and RH (+ve) and VPD (-ve), and studies on free-

1 standing Norway spruce and larch (*Larix decidua*) showed that although ambient VPD was
2 highly positively correlated with ambient O₃ concentration, increased VPD caused stomatal
3 closure, reducing O₃ uptake and impact (Wieser and Havranek, 1993, 1995).

4 Surface wetness may affect O₃ response through its direct effects on deposition to the
5 surface and through changes in RH. Effects on the deposition of O₃ have been reviewed by Cape
6 (1996). A surface film of water on leaves was found to increase O₃ deposition in four studies
7 involving field-grown grape (*Vitis vinifera*) (Grantz et al., 1995), red maple (*Acer rubrum*)
8 (Fuentes and Gillespie, 1992), deciduous forest dominated by largetooth aspen (*Populus*
9 *grandidentata*) and red maple (Fuentes et al., 1992), and clover-grass mixed pasture (*Trifolium*
10 *pratense*, *Phleum pratense*, and *Festuca pratensis*) (Pleijel et al., 1995). In each case, the
11 increased deposition could be attributed partly to an increased stomatal conductance through the
12 abaxial (lower) surface and partly to uptake into the aqueous film on the adaxial (upper) surface.
13 In contrast, decreased deposition was noted by Grantz et al. (1997) with field-grown cotton
14 (*Gossypium hirsutum*). Since cotton is amphistomatous, with functional stomata on both leaf
15 surfaces, it was suggested that, in this case, the water layer effectively sealed the adaxial surface
16 stomata, more than offsetting any increase in conductivity of the stomata in the abaxial surface.
17 However, none of the studies investigated the consequences of the differences in deposition.
18 Although it could be inferred that, with part of any increased deposition being the result of
19 increased O₃ flux into the leaves, there would be the likelihood of increased O₃ adverse effects,
20 as suggested by earlier studies (Elkiey and Ormrod, 1981) that, by misting bluegrass (*Poa*
21 *pratensis*) during exposure to O₃, injury was significantly increased.

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

25 26 **AX9.3.4.4 Drought and Salinity**

27 The 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) concluded that the
28 available evidence clearly indicated that exposure to drought conditions could reduce the adverse
29 effects of O₃ on the growth of herbaceous and woody plants, but it also noted that no quantitative
30 models of the O₃-soil moisture deficit (SMD) interaction had yet appeared in print.

31 Nevertheless, the “protective” effect was inconsistent, and only appeared when SMD was

1 accompanied by high evaporative demand. Since that time, further studies have confirmed the
2 interaction, and simulation models have begun to appear. Mills (2002) has recently provided a
3 brief review of the topic.

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

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

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

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

29 With regard to trees, O₃ interactions with soil water availability have been discussed in
30 several recent reviews: Chappelka and Freer-Smith (1995), who focused on O₃-induced

1 predisposition to drought stress (Johnson et al., 1996b; Chappelka and Samuelson, 1998; and
2 Skärby et al., 1998).

3 Several recent studies with conifers have yielded mixed results. No interactions with
4 drought were observed by Broadmeadow and Jackson (2000) on Scots pine, by Karlsson et al.
5 (2002) on Norway spruce, or by Pelloux et al. (2001) on Aleppo pine. More recently, Le Thiec
6 and Manninen (2003) reported that drought reduced O₃-induced growth suppression of Aleppo
7 pine seedlings. Panek and Goldstein (2001) inferred less impact of O₃ on droughted Ponderosa
8 pine, and Van Den Driessche and Langebartels (1994) reported that drought reduced injury and
9 O₃-induced ethylene release by Norway spruce. But Karlsson et al. (1997), in a comparative
10 study of fast- and slow-growing clones of *P. abies*, only observed a drought-induced reduction of
11 O₃-inhibited root growth in the fast-growing clone. In contrast, Grulke et al. (2002) noted a
12 synergistic interaction between O₃ and drought stress on gross photosynthesis of *Pinus*
13 *ponderosa*, and Wallin et al. (2002) reported a synergistic growth response of Norway spruce in
14 the third year of a 4-year study. A similar response was noted by Dixon et al. (1998) with the
15 Istebna strain of Norway spruce.

16 With broad-leaved trees, studies of Durmast oak (*Quercus petraea*) (Broadmeadow et al.,
17 1999; Broadmeadow and Jackson, 2000) and European ash (*Fraxinus excelsior*) (Broadmeadow
18 and Jackson, 2000; Reiner et al., 1996) showed that drought provided partial protection
19 against O₃-induced growth reduction. Although European beech (*Fagus sylvatica*) is reportedly
20 an O₃- and drought-sensitive species, neither Pearson and Mansfield (1994) nor Broadmeadow
21 et al. (1999) observed any interactions between these stresses, while Dixon et al. (1998)
22 observed partial protection. Pääkönen et al. (1998) observed only additive effects in a sensitive
23 clone of birch (*Betula pendula*). However, the experiments of Schaub et al. (2003) and the
24 survey by Vollenweider et al. (2003) on black cherry clearly indicate antagonism between
25 drought and O₃ stresses on this species.

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

30 The Plant Growth Stress Model (PGSW) developed by Chen et al. (1994) is a physiology-
31 based process model which includes drought among several environmental variables.

1 Simulations for Ponderosa pine incorporated antagonistic effects between O₃ and drought
2 stresses, i.e., partial protection, although Karlsson et al. (2000) have since emphasized that
3 drought-induced “memory effects” should be considered when developing simulation models
4 incorporating stomatal conductance.

5 Retzlaff et al. (2000) used the single-tree model, TREEGRO, to simulate the combined
6 effects of O₃ and drought on white fir (*Abies concolor*). Although simulated reductions in
7 precipitation $\geq 25\%$ reduced growth, they also reduced O₃ uptake (and impact). But lesser
8 reductions in precipitation combined synergistically with O₃ stress to reduce growth, leading the
9 authors to conclude that moderate drought may not ameliorate the response of white fir to O₃.

10 On a much larger scale with a modified forest ecosystem model (PnEt-II) incorporating
11 O₃-response relationships for hardwood species, Ollinger et al. (1997) showed how predicted
12 changes in net primary production and mean wood production in the northeastern U.S. hardwood
13 forests due to O₃ would be reduced (but not countered or reversed) by drought stress, particularly
14 in the southern part of the region. This geographic distribution of the effect was substantiated by
15 the work of Lefohn et al. (1997) on the risk to forest trees in the southern Appalachian
16 Mountains, based on localized estimates of O₃ levels and SMD. The TREEGRO and ZELIG
17 models were combined by Laurence et al. (2001) to predict the impacts of O₃ and moisture (as
18 precipitation) on the growth of loblolly pine and yellow poplar. Based on O₃ and precipitation
19 data from three sites in the eastern United States, the six model regressions developed for the
20 two species included both positive and negative coefficients for O₃ exposure and precipitation as
21 determinants of growth.

22 As noted in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996), the effects
23 of soil salinity are similar to those of SMD. In a study of rice (*Oryza sativa*) cultivars of
24 differing sensitivity to salinity, Welfare et al. (1996) noted that although both O₃ and salinity
25 reduced many features of growth additively, antagonistic interactions were only seen for leaf
26 length and potassium accumulation. Similarly, a recent study on chickpea (*Cicer arietinum*)
27 found no interactions with regard to most components of biomass accumulation (the effects
28 of O₃ and salinity were additive), but with root growth, salinity suppressed the adverse effects
29 of O₃.

30 In summary, the recently described interactions of O₃ and drought/salinity stresses are
31 consistent with the view that, in many species, drought/salinity reduces the impact of O₃, but O₃

1 increases sensitivity to drought stress, i.e., the type of response is determined by the sequence of
2 stresses. However, synergisms have also been observed and any antagonisms are species-
3 specific and unpredictable in the absence of experimental evidence. In no case has an
4 antagonism been found to provide complete protection.
5

6 **AX9.3.5 Nutritional Factors**

7 The 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) noted that the large
8 number of macro- and micronutrients and the wide range of species had limited the number of
9 experimental investigation to all but a few cases of nutrient-O₃ interactions and most of these
10 concerned nitrogen (N) and crops or forest tree species. The document also provided a
11 comprehensive tabulation of the results of the relevant studies up to 1992.

12 The suboptimal supply of mineral nutrients to plants leads to various types of growth
13 reductions. The consequences of suboptimal nutrition might, therefore, be expected to have
14 some similarities to those of O₃ exposure. One might expect nutritional levels below the
15 optimum either to amplify any effects of O₃ or at least lead to additive responses. The difficulty
16 with this suggestion is that the available information has mostly been obtained from
17 experimentation conducted using two or more arbitrarily selected levels of fertility with little or
18 no regard to optima. Hence, it is not surprising that there have been contradictory reports, even
19 among studies with the same species or cultivars conducted by different workers at different
20 locations using different soils or soil mixes.

21 There appear to have been no recent studies on O₃ interactions with specific mineral
22 nutrients other than N. Hence, the previous conclusions are still valid, viz. that increasing levels
23 of the major elements potassium (K) and sulfur (S) usually reduce the impact of O₃, or,
24 deficiency increases susceptibility, whereas increased phosphorus (P) usually increases injury,
25 or, deficiency decreases susceptibility.

26 However, with N, a relationship to the optimum is usually demonstrable. Several earlier
27 studies of O₃ × N interactions reported that the adverse effects of O₃ on growth were greatest at
28 the optimum and decreased with increasing N-deficiency, a finding supported by the work on
29 aspen of Pell et al. (1995), who also confirmed that excess N decreased O₃ impact on growth.
30 Similarly, the adverse effects of O₃ on growth rate in wheat diminished with decreased N supply

1 (Cardoso-Vilhena and Barnes, 2001). However, the effects of N are far from consistent. For
2 example, Greitner et al. (1994) reported that O₃ and N-deficiency acted additively in aspen in
3 reducing leaf surface area and rate of photosynthesis, Bielenberg et al. (2001) reported that the
4 rate of O₃-induced senescence was increased by N-deficiency in hybrid poplar (*Populus*
5 *trichocarpa* × *P. maximovizii*), and Pääkkönen and Holopainen (1995) observed the least adverse
6 effects of O₃ on European white birch (*Betula pendula*) at optimum N-fertility levels. With
7 cotton, increased N-levels more than overcame the adverse effect of O₃ on growth and boll yield
8 (Heagle et al., 1999b). In view of these contradictions, one may conclude that other, unrecorded
9 factors may have contributed to the various findings. Thus, much remains unclear about O₃ × N-
10 fertility interactions.

11 There have been two recent studies on the effects of overall soil fertility. Whitfield et al.
12 (1998) observed that low general fertility increased O₃ sensitivity in selections of common
13 plantain. At the biochemical level, well-fertilized European white birch saplings were found to
14 be less adversely affected by O₃ than nutrient-stressed plants (Landolt et al., 1997).

15 TREEGRO model simulations of the growth of red spruce (*Picea rubra*) in conditions of
16 nutrient deficiency and O₃ stress showed that, in combination, the two stresses acted less than
17 additively (Weinstein and Yanai, 1994). Minimal amelioration by nutrient deficiency was
18 predicted with Ponderosa pine.

19 Plants may also obtain N and S from airborne sources such as NO_x, HNO₃, NO₃⁻, SO₂,
20 and SO₄²⁻, although, depending upon their concentration, these may also be phytotoxic.
21 In various parts of the world, the deposition of N and S in these forms contributes significantly
22 to the levels of nutritionally available N and S in soils. Such depositions may, in turn, influence
23 the impact of O₃ on sensitive species through their roles as nutrients independent of any
24 interactions that may occur because of their acidic properties (see Section AX9.3.6.5). For
25 example, Takemoto et al. (2001) recently reviewed the situation in southern California's mixed
26 conifer forests and noted that, where N-deposition is appreciable, its combination with O₃ is
27 causing a shift in Ponderosa pine biomass allocation toward that of deciduous trees, with
28 increased needle drop so that only 1- and 2-year needle classes overwinter. Such changes are
29 having significant consequences on the balance of the forest ecosystem and are discussed more
30 fully in Section AX9.5.

1 Of the micronutrient elements, only manganese (Mn) appears to have been studied
2 recently. In beans (*Phaseolus vulgaris*) Mn-deficiency increased O₃ toxicity, despite causing
3 reduced O₃ uptake (through decreased stomatal conductance) and inducing increased levels of
4 Mn-SOD (Mehlhorn and Wenzel, 1996).

5 In view of the foregoing, it is impossible to generalize about the interactions of soil fertility
6 with O₃. While this is especially true of the interactions involving soil nitrogen, for which there
7 is much conflicting evidence, the interactions with other nutrients need much more thorough
8 investigation than has occurred to date, before any clear patterns become apparent.

9 10 **AX9.3.6 Interactions with Other Pollutants**

11 The ambient air may have pollutant gases other than O₃ and its photochemical oxidant
12 relatives. In particular, industrial, domestic, and automobile emissions and accidents can lead to
13 significant atmospheric concentrations of gases such as sulfur dioxide (SO₂) and nitric oxide
14 (NO) and nitrogen dioxide (NO₂), collectively referred to as NO_x, both locally and regionally.
15 Local releases of gases such as hydrogen fluoride (HF), hydrogen chloride (HCl), and
16 chlorine (Cl₂) may result from industrial emissions and accidents. Agricultural fertilizer and
17 manure usage can lead to significant increases in ambient ammonia (NH₃) and ammonium
18 sulfate ((NH₄)₂SO₄). The sulfur and nitrogen oxides may undergo reactions in the atmosphere
19 leading to the formation of sulphate (SO₄²⁻) and nitrate (NO₃⁻) ions and resultant acid deposition.

20 The 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) discounted much of
21 the early research on pollutant combinations, because of its lack of resemblance to the ambient
22 experience: the concentrations used were unrealistically high or the exposure regimes employed
23 almost invariably used gas mixtures, whereas Lefohn et al. (1987) showed that the co-occurrence
24 patterns of significant levels of O₃ with SO₂ or NO₂ in the United States were most frequently
25 sequential or partially sequential with overlap; only rarely were they entirely concurrent. On the
26 other hand, O₃ and peroxyacetylnitrate (PAN) frequently co-occur, as both form photochemically
27 under similar conditions.

28 To the list of reviews mentioned in the 1996 O₃ AQCD should be added the more recent
29 ones by Barnes and Wellburn (1998), Robinson et al. (1998), and Fangmeier et al. (2002), which
30 also explore some of the potential mechanisms underlying pollutant-pollutant interactions.

1 **AX9.3.6.1 Oxidant Mixtures**

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

9
10 **AX9.3.6.2 Sulfur Dioxide**

11 In reviewing O₃ × SO₂ interactions, Barnes and Wellburn (1998) remarked: “The outcome
12 of exposure to this combination of pollutants has probably been the most studied, yet is one of
13 the least understood.” More recent studies have only added to the conflicts referred to in the
14 1996 criteria document (U.S. Environmental Protection Agency, 1996), rather than resolve them.
15 For example, Diaz et al. (1996) reported that, after a year of daily exposures of Aleppo pine
16 seedlings to 50 ppb O₃ and/or 40 ppb SO₂, the combination of pollutants synergistically reduced
17 shoot and root growth and impaired mycorrhizal colonization of the roots. With tomato
18 (*Lycopersicon esculentum*), on the other hand, effects on growth ranged from synergistic at low
19 exposures (50 ppb) to antagonistic at exposures of 200 ppb of each gas (Khan and Khan, 1994).
20 Although various physiological measurements were made in these and earlier studies, it has not
21 been possible to determine any consistent mechanism or mechanisms that might account for the
22 conflicting results.

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

28
29 **AX9.3.6.3 Nitrogen Oxides, Nitric Acid Vapor, and Ammonia**

30 The major oxides of nitrogen that occur in ambient air are nitrous oxide (N₂O), NO,
31 and NO₂, of which the latter two (conveniently symbolized as NO_x) are particularly important in

1 connection with O₃, because they are components of the reaction mix that leads to photochemical
2 O₃ formation and because they can interact with O₃-responses. Their reactions in the atmosphere
3 can also lead to the occurrence of nitric acid vapor (HNO₃) in ambient air. The other major
4 N-containing contaminant of ambient air in many parts of the world is NH₃, largely released
5 through agricultural practices.

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

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

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

29 An independent case for O₃ × NO interactions comes from Mills et al. (2000). The ANN
30 model developed to predict the O₃ effects on white clover biomass based on experiments at

1 18 locations throughout Europe suggested that the minimum daily NO concentration (at 5 p.m.)
2 may have been a contributor to adverse effects.

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

7 With regard to sequential exposures, two studies on gene activation in tobacco revealed
8 that NO₂ counteracted the effect of O₃ in reducing mRNA levels for three genes encoding
9 photosynthetic proteins (Bahl and Kahl, 1995) and tended to counteract the O₃-induced
10 enhancement of defense-protein gene activation (Bahl et al., 1995). However, despite
11 compelling evidence for significant interactive effects provided by earlier studies (Bender et al.,
12 1991; Goodyear and Ormrod, 1988; Runeckles and Palmer, 1987), the only recent investigation
13 of growth effects seems to have been that of Mazarura (1997) using sine-wave exposure profiles.
14 He found that although 4 weeks of twice daily 3-h exposures to NO₂ (120 ppb peak
15 concentrations) slightly stimulated growth of radish (*Raphanus sativa*) and while daily 6-h
16 exposures to O₃ (120 ppb peak concentration) did not significantly reduce growth, the daily
17 sequence, NO₂ - O₃ - NO₂, led to a 13% drop in dry matter production.

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

28 There appear to have been no studies of O₃ interacting with HNO₃ in the vapor phase.
29 However, in the southern California montane forests (Takemoto et al., 2001), in Sweden (Janson
30 and Granat, 1999), and elsewhere, significant amounts of N are deposited in this form because of
31 the vapor's high deposition velocity. As a consequence, although much of it ultimately reaches

1 the ground through leaching and leaf fall and enters the soil as NO_3^- , it may also be used as a N
2 source by the foliage itself (Garten and Hanson, 1990; Hanson and Garten, 1992; Norby et al.,
3 1989). This nutritional role is independent of any contribution that HNO_3 vapor may make to
4 acidic deposition. Indirect interactions with the effects of O_3 through N-deposition of NO_x ,
5 HNO_3 , and NH_3 are related to the interactions of O_3 with N as a nutrient, and have recently been
6 examined in the review by Takemoto et al. (2001). The 1996 O_3 AQCD (U.S. Environmental
7 Protection Agency, 1996) stated that the evidence available at that time led to estimates of total
8 forest dry deposition, including HNO_3 , ranging from 5.7 to 19.1 kg N ha⁻¹ year⁻¹ (Taylor et al.,
9 1988). However, Takemoto et al. (2001) pointed out that in parts of the mid-elevation forests of
10 southern California, dry deposition rates may reach more than 40 kg N ha⁻¹ year⁻¹. As a result,
11 some locations have seen the conversion from N-limited to N-saturated forests. The concern for
12 California's forests is well stated by Takemoto et al.: "As potential modifiers of long-term forest
13 health, O_3 is a stressor and N deposition is an enhancer of ponderosa/Jeffrey pine physiology and
14 growth (Grulke and Balduman, 1999). The progression toward a deciduous growth habit, higher
15 shoot:root biomass ratios, increasing depths of litter, tree densification, and elevated NO_3^- levels
16 in soil and soil solution, all point to the replacement of pine species with nitrophilous, shade- and
17 O_3 -tolerant tree species, such as fir and cedar (Minnich, 1999; Minnich et al., 1995)."

18 Few studies have been reported of interactions of O_3 with NH_3 . The 1996 criteria
19 document made reference to the work on kidney bean by Tonneijck and Van Dijk (1994, 1998).
20 Although NH_3 alone tended to increase growth and O_3 alone to inhibit it, one interaction was
21 noted (Tonneijck and Van Dijk, 1994) on the number of injured leaves. Dueck et al. (1998)
22 studied the effects of O_3 and NH_3 on the growth and drought resistance of Scots pine.
23 Significant interactions were found for some growth features, but there were no consistent
24 patterns of the effects of NH_3 on O_3 response or vice versa. However, O_3 was found to
25 ameliorate the enhancement of drought stress caused by NH_3 on Scots pine.

26 At this time there is insufficient information to offer any general conclusions about the
27 interactive effects of O_3 and NH_3 .

28 29 **AX9.3.6.4 Hydrogen Fluoride and Other Gaseous Pollutants**

30 Although HF and other fluorides are important local air pollutants associated with
31 aluminum smelting and superphosphate fertilizer manufacture, no studies of possible interactions

1 with oxidants appear to have been reported since that of MacLean (1990). He found that HF
2 retarded the accelerated senescence and loss of chlorophyll resulting from O₃ exposure in corn
3 seedlings. However, such an isolated observation cannot be taken to indicate that HF can reduce
4 the impact of O₃ on other species or even that the effect would ultimately have led to an effect on
5 mature plants.

7 **AX9.3.6.5 Acidic Deposition**

8 The deposition of acidic species onto vegetation may elicit direct effects on the foliage or
9 indirect effects via changes induced in the soil. The 1996 O₃ AQCD (U.S. Environmental
10 Protection Agency, 1996) included an extensive listing of investigations into the effects of O₃
11 and acidic deposition (usually in the form of simulated acid rain, SAR) on plant growth and
12 physiology. The majority of studies found no effects of SAR or acidic mists or fogs at pH values
13 greater than about 3.0 and no interactive effects with O₃. (In ambient air, pH values less than
14 3.0 have rarely been reported.) In the few reports in which significant interactions were found,
15 most were antagonistic and were explained as probably being the result of increased fertility due
16 to NO₃⁻ and SO₄²⁻ supplied in the SAR.

17 Although numerous reviews have recently appeared (e.g., Bussotti and Ferretti, 1998;
18 Flückiger et al., 2002; Fox and Mickler, 1996; Nussbaum et al., 1999; and Sheppard and Cape,
19 1999), the shift in interest in air pollution effects away from acid deposition has resulted in little
20 new research having been reported over the past 10 or so years. In most of the reported studies,
21 no effects due to the O₃ exposures, the SAR treatments used, or their combinations were
22 observed, e.g., Baker et al. (1994) on loblolly pine; Laurence et al. (1997), and Vann et al.
23 (1995) on red spruce; and Laurence et al. (1996) on sugar maple. Branch chamber studies of
24 12-year-old Ponderosa pine trees by Momen et al. (1997, 1999) revealed no O₃ effects or
25 interactions. With red spruce, Sayre and Fahey (1999) noted no effects of O₃ on the foliar
26 leaching of Ca or Mg, which only became significant with SAR at pH 3.1. Izuta (1998)
27 observed no interactions with Nikko fir (*Abies homolepis*), although SAR at pH 4.0 reduced dry
28 matter. Shan et al. (1996) reported adverse effects of O₃ but none attributable to SAR on the
29 growth of *Pinus armandi*.

30 With herbaceous species, Ashenden et al. (1995) noted significant antagonistic interactions
31 of O₃ and acid mist in white clover, in which the adverse effect of low pH was countered by O₃.

1 In contrast, Ashenden et al. (1996) found that, although pH 2.5 mist caused a significant
2 stimulation of the growth of ryegrass attributed to a fertilizer effect, and O₃ caused reduced
3 growth, there was no interaction. Bentgrass (*Agrostis capillaris*) behaved similarly.

4 A study by Bosley et al. (1998) on the germination of spores of the moss, *Polytrichum*
5 *commune*, and the ferns, *Athyrium felix-femina* and *Onoclea sensibilis*, revealed no effect of O₃
6 on moss spores, while SAR at pH <4.0 was completely inhibitory. With the ferns, germination
7 was progressively reduced by both increased O₃ and acidity.

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

13 14 **AX9.3.6.6 Heavy Metals**

15 As there appears to have been no further research into the interactions of oxidants with
16 heavy metal pollutants, our understanding is unchanged from at the time of the 1996 O₃ AQCD
17 (U.S. Environmental Protection Agency, 1996). As noted therein, the limited data available
18 from early studies indicates varying degrees of enhancement of any adverse effects of O₃ but
19 precludes the development of any response relationships.

20 21 **AX9.3.6.7 Mixtures of Ozone with Two or More Pollutants**

22 In many airsheds, the mixtures that occur, both concurrently and over time, may involve
23 three or more pollutants. Very little useful information exists on the effects of O₃ with multiple
24 pollutants. As the 1996 criteria document and others have pointed out, most of the early studies
25 on such combinations can be discounted, because of their use of (1) high and environmentally
26 irrelevant exposure concentrations and (2) unrealistic, repetitive exposure profiles (Barnes and
27 Wellburn, 1998; U.S. Environmental Protection Agency, 1996).

28 The large investment in experimental facilities required to study these complex interactions
29 is a major deterrent. So, although the topic has been included in several reviews that have
30 appeared in the last decade, there appear to have been only two studies that have provided new
31 information on the effects of O₃ in combination with more than one other pollutant stress.

1 Ashenden et al. (1996) studied the effects of O₃ and/or (SO₂ + NO₂) with four acidities of SAR
2 applied to each gas treatment, on white clover and two pasture grasses (*Lolium perenne* and
3 *Agrostis capillaris*). With each species, the antagonism reported for the O₃ × SAR interaction
4 (Section AX9.3.4.6.5) tended to be nullified by concurrent exposure to the other gases, while the
5 combination of the three gaseous pollutants resulted in the most severe growth inhibition,
6 regardless of the acidity of the SAR.

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

10 **AX9.3.7 Interactions with Agricultural Chemicals**

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

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

26 Despite the attraction of the use of permitted chemicals to provide crop protection, the
27 statement in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) is still valid:
28 “It is premature to recommend their use specifically for protecting crops from the adverse effects
29 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 CO₂ concentrations in the lower atmosphere, and
- the effects of increasing levels of surface-level irradiation by UV-B (the result of stratospheric O₃ 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 CO₂ 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 O₃ × CO₂ interaction. Little if any experimental evidence exists related to three-way interactions, such as O₃ × CO₂ × disease or O₃ × CO₂ × nutrient availability, although such interactions cannot be predicted from the component two-way interactions.

Numerous reviews have appeared since the 1996 O₃ 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,b), McLaughlin and Percy (1999), and Saxe et al. (1998).

As background to the discussion of interactions with O₃, it should be noted that the increased levels of CO₂ 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

1 2100 (IPCC, 2001). Such increases in the concentration of CO₂, the principal GHG released into
2 the atmosphere, will inevitably lead to increased global mean temperatures, evidence for which
3 is already available from oceanic, icepack, and other records. The latest estimates of the global
4 warming are for an increase in the range of 1.4 to 5.8 °C over this century, in contrast to the
5 0.6 °C rise experienced since 1900 (IPCC, 2001). However, considerable uncertainty is
6 associated with such projections of future increases in global temperature.

7 The use of elevated CO₂ concentrations has been common practice for many years in the
8 production of many greenhouse crops. Much of our early knowledge of the effects of higher
9 than ambient CO₂ levels on plant growth derives from this application, coupled with research of
10 plant physiologists on how CO₂ concentrations affect the process of photosynthesis. Information
11 available about the effects of increased CO₂ levels on photosynthesis and stomatal function, in
12 particular, has provided the underlying bases for numerous process models that simulate plant
13 growth under stress and in changed climates.

14 Although simple O₃ × temperature interactions were discussed in Section AX9.3.4.2, the
15 close linkage between global CO₂ levels and global mean temperatures in the context of climate
16 change requires that an assessment of the interactive effects with O₃ should focus, as much as
17 possible, on interactions involving all three factors.

18 19 **AX9.3.8.1 Ozone-Carbon Dioxide-Temperature Interactions**

20 Idso and Idso (1994) reviewed several hundred reports published between 1982 and 1994
21 on the effects of increased CO₂ on plant growth and net photosynthesis. Their survey covered a
22 wide range of temperate and tropical, herbaceous and perennial species, including coniferous
23 trees. They concluded that, for responses to a 300-ppm increase in CO₂, somewhat less than a
24 doubling of present-day levels, but somewhat greater than the 540 ppm lower limit suggested by
25 the IPCC (IPCC, 2001), averaged across all species:

- 26 • light intensity had a negligible effect on net photosynthesis other than at limiting low
intensities under which the CO₂-driven enhancement was increased;
- 27 • increased temperature tended to increase the CO₂-driven enhancement of dry matter
accumulation (growth) and net photosynthesis;
- 28 • drought conditions tended to increase the CO₂-driven enhancements of both growth and
net photosynthesis, but increased salinity had little effect;

- 1 • mineral nutrient deficiency (especially of nitrogen) tended to increase the CO₂-driven enhancement of growth; and
- 2 • in the presence of air pollutants (especially SO₂ and NO_x), the CO₂-driven enhancement of net photosynthesis tended to be increased.

3 It should be noted that the statement that CO₂-enhanced growth increased with temperature
4 referred to total dry matter accumulation by the whole plant and not to the yield of grain, fruit, or
5 seed. Unfortunately, despite the existence of several reports at the time, the summary of
6 interactions with air pollutants contained only a single reference to O₃, i.e., Pfirrmann and
7 Barnes (1993), who reported surprisingly that a doubling of CO₂ levels led to a 27% increase in
8 dry weight of radish but that the combination with O₃ led to a 77% increase.

9 The more recent reviews by Rudorff et al. (2000) and Olszyk et al. (2000) have
10 addressed CO₂ × O₃ interactions in detail, with the latter focusing on the implications for
11 ecosystems. They concluded that:

- 12 • the effects of both gases on stomatal closure were predominantly additive, with little
evidence of interaction;
- 13 • increased photosynthesis resulting from elevated CO₂ may be canceled by exposures to
high O₃ levels;
- 14 • foliar O₃ injury is reduced by elevated CO₂; and
- 15 • interactions between CO₂ and O₃ can affect storage carbohydrates, leaf free-radical
metabolism, and carbon allocation to shoots and roots.

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

18 Much of the recently published information on the effects of increased CO₂ and O₃ levels
19 is summarized in Table AX9-12. Note that the table only lists the *directions* of O₃-induced
20 effects and any modifications of these effects resulting from elevated CO₂, not their magnitudes.
21 These directions are usually, but not necessarily, the same as the corollary effects of O₃
22 on CO₂-induced responses.

23 The bulk of the available evidence clearly shows that, under the various experimental
24 conditions used (which almost exclusively employed abrupt or “step” increases in CO₂
25 concentration, as discussed below), increased CO₂ levels may protect plants from the adverse

Table AX9-12. Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

O ₃ Effects: ∇, Decrease; ∧, Increase; ○, No Significant Effect. CO ₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Biochemical/Metabolic</i>					
Ascorbate peroxidase	∇	▼	Wheat (<i>T. aestivum</i>)	CSTR, P	Rao et al. (1995)
	∇	○□	Sugar maple (<i>A. saccharum</i>)	CEC, P	Niewiadomska et al. (1999)
	∇	○□	Trembling aspen (<i>P. tremuloides</i>)	FACE, G	Wustman et al. (2001)
Catalase	∇	○□	Wheat (<i>T. aestivum</i>)	CEC, P	McKee et al. (1997b)
	∧	○□		CEC, P	Niewiadomska et al. (1999)
	∇	○□	Soybean (<i>G. max</i>)	OTC, P	Booker et al. (1997)
Chlorophyll	∇	▼	Wheat (<i>T. aestivum</i>)	OTC, G	Donnelly et al. (2000); Ommen et al. (1999)
	∇	▼	Potato (<i>S. tuberosum</i>)	OTC, G	Donnelly et al. (2001a)
	∇	▼	Soybean (<i>G. max</i>)		Heagle et al. (1998); Reid and Fiscus (1998); Reid et al. (1998)
Glutathione reductase	∇	▼	Wheat (<i>T. aestivum</i>)	CSTR, P	Rao et al. (1995)
	○	○□	Sugar maple (<i>A. saccharum</i>)	CEC, P	Niewiadomska et al. (1999)
	○	○□	Aspen (<i>P. tremuloides</i>)	FACE, G	Wustman et al. (2001)
Glycolate oxidase	∇	▼	Soybean (<i>G. max</i>)	OTC, P	Booker et al. (1997)
Hydroxypyruvate reductase	∇	○□	Soybean (<i>G. max</i>)	OTC, P	Booker et al. (1997)
Rubisco	∇	▼	Soybean (<i>G. max</i>)	OTC, P	Reid et al. (1998)
	∇	▼	Wheat (<i>T. aestivum</i>)	OTC, G	McKee et al. (2000)
	[∧]	▼	Trembling aspen (<i>P. tremuloides</i>)	FACE, G	Noormets et al. (2001)
	∇	▼	Sugar maple (<i>A. saccharum</i>)	CEC, P	Gaucher et al. (2003)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

<p style="text-align: center;">O₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect. CO₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○□, No Significant Effect.</p>					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Biochemical/Metabolic (cont'd)</i>					
Rubisco activity	∨	▼	Soybean (<i>G. max</i>)	OTC, P	Reid et al. (1998)
	∨	▼	Wheat (<i>T. aestivum</i>)	CEC, P	McKee et al. (1995)
	∨	[▼]	Wheat (<i>T. aestivum</i>)	OTC, G	McKee et al. (2000)
	∨	▼	European beech (<i>F. sylvatica</i>)	CEC, P	Lütz et al. (2000)
Superoxide dismutase	○	○□	Wheat (<i>T. aestivum</i>)	CEC, P	McKee et al. (1997b)
	○	○□	Sugar maple (<i>A. saccharum</i>)	CEC, P	Niewiadomska et al. (1999)
Physiological					
Stomatal conductance	∨	▲	Radish (<i>R. sativus</i>)	CEC, P	Barnes and Pfirrmann (1992)
	∨	▼	Soybean (<i>G. max</i>)	OTC, G	Mulchi et al. (1992)
	∨	▲	Soybean (<i>G. max</i>)	OTC, P,G	Booker et al. (2005); Fiscus et al. (1997)
	∨	▼	Bean (<i>P. vulgaris</i>)	OTC, P	Heagle et al. (2002)
	∨	[▲]	White clover (<i>T. repens</i>) (O ₃ -sensitive)	CSTR, P	Heagle et al. (1993)
	∧ - ○□	▼ - ○□	White clover (<i>T. repens</i>) (O ₃ -tolerant)	CSTR, P	Heagle et al. (1993)
	∨	▲	Tomato (<i>L. esculentum</i>)	CEC, P	Hao et al. (2000)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

O ₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect. CO ₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Physiological (cont'd)</i>					
Stomatal conductance (cont'd)	∨	▲	Potato (<i>S. tuberosum</i>)	OTC, G	Finnan et al. (2002)
	∨	▲	Wheat (<i>T. aestivum</i>)	CEC, P	Balaguer et al. (1995) Barnes et al. (1995)
	[∨]	▲		CEC, P	McKee et al. (1995)
	∨	○□		OTC, G	Mulholland et al. (1997b)
	∨	○ - ▲		CEC, P	Donnelly et al. (1998)
	∧	○□		CEC, P	Tiedemann and Firsching (2000)
	∨	○□	<i>Agropyron smithii</i>	CEC, P	Volin et al. (1998)
	∨	○□	<i>Koeleria cristata</i>	CEC, P	Volin et al. (1998)
	○□	○□	<i>Bouteloua curtipendula</i>	CEC, P	Volin et al. (1998)
	○□	○□	<i>Schizachyrium scoparium</i>	CEC, P	Volin et al. (1998)
	○□	○□	Black cherry (<i>P. serotina</i>)	CSTR, P	Loats and Rebbeck (1999)
	○□	○□	Green ash (<i>F. pennsylvanica</i>)	CSTR, P	Loats and Rebbeck (1999)
	[∧]	▼	Yellow poplar (<i>L. tulipifera</i>)	CSTR, P	Loats and Rebbeck (1999)
	∨	▲	Trembling aspen (<i>P. tremuloides</i>)	CEC, P	Volin et al. (1998)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

O ₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect.					
CO ₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Physiological (cont'd)</i>					
Stomatal conductance (cont'd)	○ - ∨	▼		FACE, G	Noormets et al. (2001)
	○□	○□	Red oak (<i>Q. rubra</i>)	CEC, P	Volin et al. (1998)
	[∧]	▼	Durmast oak (<i>Q. petraea</i>)	CEC, P	Broadmeadow et al. (1999)
Photosynthesis	∨	▼	Radish (<i>R. sativus</i>)	CEC, P	Barnes and Pfirrmann (1992)
	∨	▼	Soybean (<i>G. max</i>)	OTC, P	Booker et al. (1997)
	∨	▼	Soybean (<i>G. max</i>)	OTC, G,P	Mulchi et al. (1992)
	∨	▼	Bean (<i>P. vulgaris</i>)	OTC, P	Heagle et al. (2002)
	∨	○□	Cotton (<i>G. hirsutum</i>)	OTC, P	Heagle et al. (1999b)
	∨	○□	Tomato (<i>L. esculentum</i>)	CEC, P	Hao et al. (2000)
	○□	○□	Potato (<i>S. tuberosum</i>)	OTC, G	Donnelly et al. (2001a)
	○□	[▼]		OTC, G	Lawson et al. (2001b)
	[∨]	▼	Wheat (<i>T. aestivum</i>)	CEC, P	Barnes et al. (1995)
	∨	▼	Wheat (<i>T. aestivum</i>)	OTC, G	Donnelly et al. (2000); Mulholland et al. (1997b) Reid and Fiscus (1998)
				CEC, P	Tiedemann and Firsching (2000)
	∨	○□		CEC, P	Cardoso-Vilhena and Barnes (2001)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

<p style="text-align: center;">O₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect. CO₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.</p>					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Physiological (cont'd)</i>					
Photosynthesis (cont.)	○	○	<i>Agropyron smithii</i>	CEC, P	Volin et al. (1998)
	∨	▼	<i>Koeleria cristata</i>	CEC, P	Volin et al. (1998)
	○ - ∨	○	<i>Bouteloua curtipendula</i>	CEC, P	Volin et al. (1998)
	[∨]	▼	<i>Schizachyrium scoparium</i>	CEC, P	Volin et al. (1998)
	∨	▼	Ponderosa pine (<i>P. ponderosa</i>)	CEC, G	Olszyk et al. (2001)
	∨	▼	Scots pine (<i>P. sylvestris</i>)	OTC, G	Kellomäki and Wang (1997a,b)
	○	○	Black cherry (<i>P. serotina</i>)	CSTR, P	Loats and Rebbeck (1999)
	[∨]	▼	Green ash (<i>F. pennsylvanica</i>)	CSTR, P	Loats and Rebbeck (1999)
	○	○	Yellow poplar (<i>L. tulipifera</i>)	CSTR, P	Loats and Rebbeck (1999)
	∨	▼	Trembling aspen (<i>P. tremuloides</i>)	CEC, P	Volin et al. (1998)
	∨	▼	European beech (<i>F. sylvatica</i>)	CEC, P	Grams et al. (1999)
	○	○	Red oak (<i>Q. rubra</i>)	CEC, P	Volin et al. (1998)
	∨	▼	Sugar maple (<i>A. saccharum</i>)	CEC, P	Gaucher et al. (2003)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

<p style="text-align: center;">O₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect. CO₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.</p>					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Physiological (cont'd)</i>					
Photorespiration	∧	▼	Soybean (<i>G. max</i>)	OTC, P	Booker et al. (1997)
	∨	▲	Wheat (<i>T. aestivum</i>)	CEC, P	McKee et al. (1997b)
<i>Growth, Yield</i>					
Total biomass	∨	▼	Parsley (<i>P. sativum</i>)	CEC, P	Cardoso-Vilhena et al. (1998)
	∨	▼	Bean (<i>P. vulgaris</i>)	CEC, P	Cardoso-Vilhena et al. (1998)
	∨	▼		OTC, P	Heagle et al. (2002)
	∨	▼	Soybean (<i>G. max</i>)	OTC, G CSTR, P OTC, P,G OTC, P	Mulchi et al. (1992) Reinert et al. (1997) Booker et al. (2005) Booker et al. (2004)
	[∨]	○	Alfalfa (<i>M. sativa</i>)	CEC, P	Johnson et al. (1996a)
	∨	▼	White clover (<i>T. repens</i>) (O ₃ -sensitive)	CSTR, P	Heagle et al. (1993)
	○	○	White clover (<i>T. repens</i>) (O ₃ -tolerant)	CSTR, P	Heagle et al. (1993)
	∨	▼	Tomato (<i>L. esculentum</i>)	CEC, P CSTR, P	Hao et al. (2000) Reinert and Ho (1995)
	○	○	Potato (<i>S. tuberosum</i>)	OTC, G	Donnelly et al. (2001b); Persson et al. (2003)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

O ₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect.					
CO ₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Growth, Yield (cont'd)</i>					
Total biomass (cont'd)	∨	○□		OTC, G	Lawson et al. (2001a)
	∨	▼	Mustard (<i>S. alba</i>)	CEC, P	Cardoso-Vilhena et al. (1998)
	∨	▼	Plantain (<i>P. major</i>)	CEC, P	Cardoso-Vilhena et al. (1998)
	∨	▼	Cotton (<i>G. hirsutum</i>)	OTC, P	Booker (2000) Heagle et al. (1999b)
	∨	▼	Wheat (<i>T. aestivum</i>)	CEC, P OTC, G OTC, P CEC, P OTC, G CSTR, P OTC, G	Cardoso-Vilhena et al. (1998) Fangmeier et al. (1996) Heagle et al. (2000) McKee et al. (1997a) Pleijel et al. (2000) Rao et al. (1995) Rudorff et al. (1996a)
	[∨]	▼	Wheat (<i>T. aestivum</i>)	OTC, G	Bender et al. (1999) Mulholland et al. (1997a)
	∨	○□	Wheat (<i>T. aestivum</i>)	CEC, P	Cardoso-Vilhena et al. (1998) Tiedemann and Firsching (2000)
	∨	[▼]	Wheat (<i>T. aestivum</i>)	OTC, G	Ewart and Pleijel (1999)
	[∨]	▼	Timothy (<i>P. pratense</i>)	CEC, P	Johnson et al. (1996a)
	∨	▼	<i>Agropyron smithii</i>	CEC, P	Volin et al. (1998)
	∨	▼	<i>Koeleria cristata</i>	CEC, P	Volin et al. (1998)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

O ₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect. CO ₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Growth, Yield (cont'd)</i>					
Total Biomass (cont'd)	∨	▼	Corn (<i>Z. mays</i>)	OTC, G	Rudorff et al. (1996a)
	∨	○□	<i>Bouteloua curtipendula</i>	CEC, P	Volin et al. (1998)
	○□	○□	<i>Schizachyrium scoparium</i>	CEC, P	Volin et al. (1998)
	∨	▼	Ponderosa pine (<i>P. ponderosa</i>)	CEC, G	Olszyk et al. (2001)
	∨	▲	Birch (<i>B. pendula</i>)	CEC, P	Kytöviita et al. (1999)
	[∨]	○□	Black cherry (<i>P. serotina</i>)	CSTR, P	Loats and Rebbeck (1999)
	∧	▼	Green ash (<i>F. pennsylvanica</i>)	CSTR, P	Loats and Rebbeck (1999)
	○□	○□	European ash (<i>F. excelsior</i>)	OTC, G	Broadmeadow and Jackson (2000)
	[∧]	▲	Yellow poplar (<i>L. tulipifera</i>)	CSTR, P	Loats and Rebbeck (1999)
	∨	▼	Sugar maple (<i>A. saccharum</i>)	CEC, P	Gaucher et al. (2003)
	∨	▼	Trembling aspen (<i>P. tremuloides</i>) (O ₃ -tolerant clone}	CEC, P OTC, P OTC, G	Volin et al. (1998) Dickson et al. (1998) Dickson et al. (2001)
	∨	○□	Trembling aspen (<i>P. tremuloides</i>) (O ₃ -sensitive clone)	OTC, G	Dickson et al. (2001)
	○□	○□	Red oak (<i>Q. rubra</i>)	CEC, P	Volin et al. (1998)
	∨	▼	Durmast oak (<i>Q. petraea</i>)	CEC, P OTC, G	Broadmeadow et al. (1999) Broadmeadow and Jackson (2000)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

O ₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect.					
CO ₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Growth, Yield (cont'd)</i>					
Total Biomass (cont'd)	○□	○□	Aleppo pine (<i>P. halepensis</i>)	CEC, P	Kytöviita et al. (1999)
	○□	○□	Scots pine (<i>P. sylvestris</i>)	OTC, G	Broadmeadow and Jackson (2000)
Seed/grain/fruit/tuber yield	∨	▼	Soybean (<i>G. max</i>)	OTC, P OTC, G OTC, G OTC, P,G	Fiscus et al. (1997) Mulchi et al. (1992) Mulchi et al. (1995) Booker et al. (2005)
	∨	▼	Bean (<i>P. vulgaris</i>)	OTC, P	Heagle et al. (2002)
	∨	▼	Tomato (<i>L. esculentum</i>)	CSTR, P	Reinert and Ho (1995)
	∨	▼	Potato (<i>S. tuberosum</i>)	OTC, G	Finnan et al. (2002)
	○□	○□		OTC, G	Persson et al. (2003)
	∨	▼	Wheat (<i>T. aestivum</i>)	OTC, G OTC, G CEC, P OTC, G OTC, G OTC, G	Bender et al. (1999) Fangmeier et al. (1996); McKee et al. (1997a) Mulchi et al. (1995) Mulholland et al. (1998b) Rudorff et al. (1996b)
	[∨]	▼	Wheat (<i>T. aestivum</i>) (cont.)	OTC, G	Fangmeier et al. (1996)
	∨	[▼]		OTC, G	Mulholland et al. (1998b); Mulholland et al. (1998a)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

<p style="text-align: center;">O₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect. CO₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.</p>					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Growth, Yield (cont'd)</i>					
Relative growth rate	∨	▼	Wheat (<i>T. aestivum</i>)	CEC, P	Barnes et al. (1995)
	∨	▼		CEC, P	Cardoso-Vilhena and Barnes (2001)
	∨	▼	<i>Agropyron smithii</i>	CEC, P	Volin et al. (1998)
	∨	▼	<i>Koeleria cristata</i>	CEC, P	Volin et al. (1998)
	○□	○□	<i>Bouteloua curtipendula</i>	CEC, P	Volin et al. (1998)
	○□	○□	<i>Schizachyrium scoparium</i>	CEC, P	Volin et al. (1998)
	○□	○□	European ash (<i>F. excelsior</i>)	OTC, G	Broadmeadow and Jackson (2000)
	∨	▼	Trembling aspen (<i>P. tremuloides</i>)	CEC, P	Volin et al. (1998)
	[∨]	▼	Red oak (<i>Q. rubra</i>)	CEC, P	Volin et al. (1998)
	∨	▼	Durmast oak (<i>Q. petraea</i>)	OTC, G	Broadmeadow and Jackson (2000)
	○□	○□	Scots pine (<i>P. sylvestris</i>)	OTC, G	Broadmeadow and Jackson (2000)
Specific leaf area-SLA	∨	○□	Radish (<i>R. sativus</i>)	CEC, P	Barnes and Pfirrmann (1992)
	∨	▼	Soybean (<i>G. max</i>)	OTC, P	Reid et al. (1998)
	∨	▼	Cotton (<i>G. hirsutum</i>)	OTC, P	Booker (2000)
	∨	○□		OTC, G	Mulchi et al. (1992)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

<p style="text-align: center;">O₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect. CO₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.</p>					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Growth, Yield (cont'd)</i>					
Specific leaf area-SLA (cont'd)	∨	▲	White clover (<i>T. repens</i>) (O ₃ -sensitive)	CSTR, P	Heagle et al. (1993)
	∧	▲	White clover (<i>T. repens</i>) (O ₃ -tolerant)	CSTR, P	Heagle et al. (1993)
	∧	▲	<i>Agropyron smithii</i>	CEC, P	Volin et al. (1998)
	∧	▲	<i>Koeleria cristata</i>	CEC, P	Volin et al. (1998)
	○	○	<i>Bouteloua curtipendula</i>	CEC, P	Volin et al. (1998)
	∧	○	<i>Schizachyrium scoparium</i>	CEC, P	Volin et al. (1998)
	∧	▲	Trembling aspen (<i>P. tremuloides</i>)	CEC, P	Volin et al. (1998)
	∧	▲	Red oak (<i>Q. rubra</i>)	CEC, P	Volin et al. (1998)
Root/shoot ratio	∨	▼	Radish (<i>R. sativus</i>)	CEC, P	Barnes and Pfirmann (1992)
	∨	▲	Alfalfa (<i>M. sativa</i>)	CEC, P	Johnson et al. (1996a)
	∨	▼	White clover (<i>T. repens</i>) (O ₃ -sensitive)	CSTR, P	Heagle et al. (1993)
	○	○	White clover (<i>T. repens</i>) (O ₃ -tolerant)	CSTR, P	Heagle et al. (1993)
	∧	▲	Wheat (<i>T. aestivum</i>)	CEC, P	McKee et al. (1997a)

Table AX9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

O ₃ Effects: √, Decrease; ∧, Increase; ○, No Significant Effect.					
CO ₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○□, No Significant Effect.					
Plant Response	O ₃ Response ^a	CO ₂ Modification ^b	Species	Facility ^c	Reference
<i>Growth, Yield (cont'd)</i>					
Root/shoot ratio (cont'd)	√	▼	Timothy (<i>P. pratense</i>)	CEC, P	Johnson et al. (1996a)
	√	○□	Soybean (<i>G. max</i>)	OTC, G,P	Booker et al. (2005)
	○□	○□	Black cherry (<i>P. serotina</i>)	CSTR, P	Loats and Rebbeck (1999)
	○□	○□	Green ash (<i>F. pennsylvanica</i>)	CSTR, P	Loats and Rebbeck (1999)
	○□	○□	Yellow poplar (<i>L. tulipifera</i>)	CSTR, P	Loats and Rebbeck (1999)
	○□	○□	Aspen (<i>P. tremuloides</i>)	OTC, G	Dickson et al. (2001)
Foliar injury	∧	▼	Potato (<i>S. tuberosum</i>)	OTC, G	Donnelly et al. (2001b) Persson et al. (2003)
	∧	▼	Bean (<i>P. vulgaris</i>)	OTC, P	Heagle et al. (2002)
	∧	▼	Soybean (<i>G. max</i>)	OTC, P	Heagle et al. (1998)
	∧	▼	Cotton (<i>G. hirsutum</i>)	OTC, P	Heagle et al. (1999b)
	∧	▼	Wheat (<i>T. aestivum</i>)	CEC, P OTC, G	Barnes et al. (1995) Mulholland et al. (1997a)
	∧	▼	Trembling aspen (<i>P. tremuloides</i>)	FACE, G	Karnosky et al. (1999) Wustman et al. (2001)
	∧	▼	European beech (<i>F. sylvatica</i>)	CEC, P	Grams et al. (1999)

O₃ m ≤ 0.15 ppm.

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

^c 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*.

1 effects of O₃ on growth. This protection may be afforded in part by CO₂ acting together with O₃
2 in inducing stomatal closure, thereby reducing O₃ uptake, and in part by CO₂ reducing the
3 negative effects of O₃ on Rubisco and its activity in CO₂-fixation. Although both CO₂-induced
4 and O₃-induced decreases in stomatal conductance have been observed primarily in short-term
5 studies, recent data clearly show a long-term and sustained reduction in stomatal conductance
6 under elevated CO₂ for a number of species (Ainsworth and Long, 2005; Ellsworth et al., 2004;
7 Gunderson et al., 2002). Instances of increased stomatal conductance have also been observed in
8 response to O₃ exposure, suggesting partial stomatal dysfunction after extended periods of
9 exposure (Maier-Maercker, 1998).

10 At the mechanistic level, Rubisco plays a key role in CO₂-assimilation, and while both O₃
11 and elevated CO₂ per se can lead to reduced activity, CO₂ can also reverse the O₃-induced
12 inhibition of Rubisco activity and photosynthesis (Table AX9-12). However, in their review of
13 the possible mechanisms involved, Polle and Pell (1999) cautioned that Rubisco should not be
14 regarded “as a unique target for the interaction of the two gases.” But it is clear from the bulk of
15 the evidence in Table AX9-12 that elevated CO₂ levels can ameliorate the inhibition of growth
16 caused by O₃ in many species, although the precise balance among the mechanisms involved
17 may well vary from species to species. Three important caveats must be raised with regard to
18 the findings presented in Table AX9-12:

- 19 ● the applicability of results from experiments with an abrupt (step) increase in CO₂ level
to understanding the consequences of the gradual increase in CO₂ predicted for the
troposphere over the next hundred years;
- 20 ● 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
- 21 ● the relevance to understanding the effects of climate change of studies focused solely on
CO₂ 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 CO₂ (Intergovernmental Panel on Climate Change
(IPCC), 2001).

22 The first caveat concerns the distinctly different natures of the exposures to O₃ and CO₂
23 experienced by plants in the field. Changes in the ambient concentrations of these gases have
24 very different dynamics. In the context of climate change, CO₂ levels increase relatively slowly

1 and may change little over several seasons of growth. On the other hand, O₃ presents a
2 fluctuating stressor with considerable hour-to-hour and day-to-day variability (Polle and Pell,
3 1999). Almost all of the evidence presented in Table AX9-12 comes from experimentation
4 involving plants grown from the outset in, or subjected to, an abrupt or step increase to a higher
5 (more or less double), steady CO₂ concentration. In contrast, the O₃ exposure concentrations
6 usually varied from day to day. Luo and Reynolds (1999), Hui et al. (2002), and Luo (2001)
7 noted the difficulties in predicting the likely effects of a gradual CO₂ increase from experiments
8 involving a step increase or those using a range of CO₂ concentrations. Indeed, although using
9 the much accelerated timescale of an 80-day growing season, Hui et al. (2002) clearly showed
10 significant differences between the rates and magnitudes of various physiological and growth
11 responses of plantain (*Plantago lanceolata*) to CO₂ between gradual and step increase
12 treatments. The authors concluded that, even though there were major differences in most of the
13 parameters studied between the gradual and step treatments, “the convergence of the measured
14 parameters at the end of the experiment provides some encouragement for the applicability of
15 step-type experiments in the field; however, the study suggests caution in interpreting early
16 results from short-term studies.”

17 In long-term studies, the matter of photosynthetic acclimation to elevated CO₂ levels has to
18 be considered. Lawlor and Keys (1993) define *acclimation* in terms of long-term (days, weeks),
19 irreversible physiological changes, in contrast to *regulation*, which relates to more rapid
20 (minutes, hours), reversible changes. Each may be positive or negative, but many studies
21 indicate that, while positive acclimation to elevated CO₂ levels initially led to enhanced
22 photosynthesis and growth, negative acclimation ultimately ensued and reduced CO₂
23 assimilation and growth rates. However, the consensus from recent studies and reviews is that
24 such negative acclimation is most likely to occur in situations in which plants are grown under
25 some additional stress, induced, e.g., by limitations to growth posed by lack of resources such as
26 water or nutrients. The meta-analysis by Curtis (1996) revealed that slow or little negative
27 acclimation was noted in studies on unstressed tree species with unhindered opportunities for
28 root growth and development, a view originally suggested by Arp and Drake (1991) and largely
29 supported in the review by Eamus (1996). A nonwoody perennial, the rhizomatous wetland
30 sedge, *Scirpus olneyi*, grown in its natural environment with no edaphic limitations showed no
31 negative acclimation after 4 years; in fact, photosynthetic capacity increased by 31% (Arp and

1 Drake, 1991). No negative acclimation of well-watered, field-grown Ponderosa pine trees was
2 observed by Tissue et al. (1999) after 6 years of growth at 2×-ambient CO₂ levels. Gifford and
3 Morison (1993) have summarized the situation thus: “Where the aerial or root environment for a
4 plant is restricted (as with inter-plant competition, for example), positive feedback is limited and
5 adjustments to the changed resource input balance under high CO₂ can include ‘down-
6 regulation’ of leaf photosynthesis rate as an integral part of a positive growth response.”

7 The influence of other environmental stressors is borne out by several long-term tree
8 studies. After 3 years in 565 ppm CO₂ in the Duke Forest FACE facility in North Carolina,
9 maturing loblolly pine trees showed only a marginal CO₂-induced carbon gain if grown on a
10 nutritionally moderate site, but zero gain if grown on a nutritionally poor site (Oren et al., 2001).
11 This is in sharp contrast to the substantially increased initial growth rates in elevated CO₂
12 reported by DeLucia et al. (1999), but it is supported by the observations of Tognetti et al.
13 (2000) on five Mediterranean tree species growing for many years adjacent to geothermal
14 springs releasing CO₂ sufficient to provide ambient levels averaging 700 ppm. No significant
15 differences in radial growth of the oaks (*Quercus cerris*, *Q. ilex*, and *Q. pubescens*), strawberry
16 tree (*Arbutus unedo*), and flowering ash (*Fraxinus ornus*) could be detected between trees at the
17 naturally enriched site and those at a nearby site exposed to normal ambient CO₂ (~350 ppm).
18 The authors concluded that limited availability of water and nutrients may have counteracted any
19 positive effects of CO₂ on growth at the enriched site or that the trees had acclimated to the
20 higher CO₂ levels.

21 Because the ameliorative effects of CO₂ on responses to O₃ (Table AX9-14) were reported
22 mostly in short-term studies involving an abrupt increase in CO₂ level, it is appropriate to ask
23 whether this amelioration is likely to persist to a time when the ambient CO₂ concentration is
24 relatively stable at such levels. Regardless of any negative acclimation due to resource
25 limitations that may occur in the interim, steadily rising CO₂ levels may well lead to natural
26 selection and genetic change. Nevertheless, it seems reasonable to expect that the amelioration
27 of O₃ impact at elevated CO₂ levels will be maintained in many situations, but the negative
28 acclimation that will probably occur in situations where other resources become limiting will
29 reduce the degree of protection.

30 Another caveat regarding the validity of some of the observations in Table AX9-12 is
31 related to the matter of stress-induced negative acclimation to elevated CO₂ and concerns related

1 to using potted plants. Although much of the recent information on CO₂ effects has come
2 from experiments with plants rooted in the ground, more than half of the studies listed in
3 Table AX9-12 used potted plants, whether in controlled environment and greenhouse chambers
4 or in OTCs. The degree to which pot-based studies resulted in similar patterns of response to
5 soil-grown plants appears to depend on the treatment conditions and plant growth conditions
6 used in the study. The use of potted plants was a confounding factor in the studies of Taylor
7 et al. (2001) of the differences in leaf growth of poplar (*Populus*) hybrids between plants
8 exposed to elevated CO₂ in controlled environment chambers (potted plants), OTCs, or a FACE
9 facility. Loats and Rebbeck (1999) suggested that their lack of CO₂ response in three broadleaf
10 species may have resulted from their use of pot-grown plants. In contrast, Heagle et al. (1999a)
11 found that the relative enhancement of soybean photosynthesis, growth, and yield by CO₂
12 enrichment was similar in pots and in the ground. These findings were supported by Booker
13 et al. (2005). The recent meta-analysis of data on the effects of elevated CO₂ on soybean
14 physiology and growth by Ainsworth et al. (2002) revealed a threefold smaller stimulation of
15 seed yield in pot-grown than in field-grown plants, even when large (>9 L) pots were used.
16 However, Ainsworth et al. (2002) included a wide range of treatment conditions (e.g., CO₂
17 treatments ranging from 500 to 1200 ppm) and plant growth conditions in the meta-analysis, so
18 caution is needed when generalizing conclusions about the applicability of pot-based studies.

19 Although the majority of the cases cited in Table AX9-12 indicate that O₃ and CO₂ act
20 additively or synergistically in causing stomatal closure, there are numerous exceptions.
21 Any reduction in stomatal aperture has consequences other than merely restricting O₃ uptake and
22 the exchange of other gases. In particular, stomatal closure initially reduces the rate of
23 transpiration, although increased leaf temperature and VPD associated with stomatal closure can
24 offset decreases in transpiration. In instances where transpiration is reduced, water-use
25 efficiency may increase, however decreased transpirational flux may lead to decreased mineral
26 uptake, which could adversely impact growth over extended periods.

27 Hence, the final caveat regarding Table AX9-12 concerns the interactions of O₃ and CO₂
28 with other climatic variables, especially mean temperature. In light of the key role played
29 by temperature in regulating physiological processes and modifying plant response to
30 increased CO₂ levels (Long, 1991; Morison and Lawlor, 1999) and the knowledge that
31 relatively modest increases in temperature may lead to dramatic consequences in terms of

1 plant development (Lawlor, 1998), it is unfortunate that much of the large investment in time
2 and resources spent on recent studies of the effects of climate change on vegetation have gone
3 into investigations limited to increasing our knowledge of the effects of higher levels of CO₂ *at*
4 *current ambient temperatures*.

5 Some attention is now being paid to investigating the concurrent effects of CO₂ increases
6 and warming (recently reviewed by Rowland-Bamford [2000] and Morison and Lawlor [1999]),
7 but the observed interactive effects on plant growth are inconsistent. For example, a FACE
8 study with ryegrass (*Lolium perenne*) showed that increased temperatures (provided by infrared
9 heaters) reduced the dry matter gain resulting from increased CO₂ levels (Nijs et al., 1996). The
10 field studies by Shaw et al. (2002) on a California annual grassland dominated by the grasses
11 *Avena barbata* and *Bromus hordeaceus* and the forbs *Geranium dissectum* and *Erodium botrys*
12 involved free-air increased CO₂ as well as increased temperature, precipitation, and N supply.
13 Not only did increased temperature reduce CO₂-stimulated net primary productivity (NPP), but
14 increased CO₂ itself, combined with other factors, was found to be able to reduce NPP.

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

1 An indirect affirmation of the importance of temperature as a component of climate change
2 on wheat yield was provided by Van Oijen and Ewart (1999) using two simulation models,
3 AFRCWHEAT2-O₃ and LINTULCC (Ewart et al., 1999). They analyzed data from the
4 ESPACE-wheat program, which involved 25 OTC experiments in 1994, 1995, and 1996 at nine
5 European locations (Jäger et al., 1999). Both models were able to predict control-treatment grain
6 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),
7 and both indicated a predominantly negative effect of temperature on the yield response to
8 increased CO₂ (a 3 °C rise reduced the gain in yield from 30 to 14%). However, neither model
9 had an R² > 0.3, indicating that the models included other sources of variability among the sites
10 than the climatic factors. The multiple linear regression developed by Bender et al. (1999) based
11 on the same datasets also included temperature as a highly significant covariant. Both studies
12 are discussed more fully below.

13 Other studies, however, have found positive temperature-related growth effects, as
14 suggested by the early Idso and Idso (1994) analysis. In an OTC study using the perennial
15 grass *Festuca pratensis* in which a temperature increase of 3 °C above ambient was combined
16 with CO₂-enrichment to 700 ppm, both CO₂ and temperature caused increases in total above-
17 ground biomass (Hakala and Mela, 1996). Studies with potato (Cao et al., 1994) and soybean
18 (Ziska and Bunce, 1997) using potted plants in controlled environment chambers also showed
19 temperature-enhanced increases in growth in enriched CO₂ atmospheres. Read and Morgan
20 (1996) compared the effects of enriched CO₂ and temperature on two grasses: cool-season
21 *Pascopyrum smithii* and warm-season *Bouteloua gracilis*. In the latter (a C₄ species),
22 750 ppm CO₂ resulted in increased dry matter production at daytime temperatures as high as
23 35 °C, but in *P. smithii* (a C₃ species), CO₂-stimulated growth was greatest at 20 °C. However,
24 the stimulation was progressively attenuated by increased temperature, so that at 35 °C, growth
25 in 750 ppm was only one third of that in 350 ppm CO₂ at 20 °C.

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

30 But what if we add O₃ as another variable? Unfortunately, there have been very few
31 studies of the three-way interaction. With the information available on CO₂ × O₃ interactions

1 (Table AX9-12) and the limited information on temperature \times O₃ interactions (discussed in
2 Section AX9.3.4.2) simulation modeling can attempt to provide estimates of O₃ \times CO₂ \times
3 temperature effects, but experimental observation is still required to validate the models. The
4 questions that need to be answered are: if increased temperature can offset the gains in
5 productivity afforded by increased CO₂ in important species such as wheat, and increased CO₂
6 can offset the reductions in productivity caused by O₃, will increased temperature modify this
7 protective effect? And if so, in what manner?

8 To date, the only information available appears to consist of the reports by Van Oijen and
9 Ewart (1999) and Bender et al. (1999) referred to above. In the former's simulation studies, the
10 overall yield depression of wheat caused by O₃ was found to be $7 \pm 4\%$ for both
11 AFRCWHEAT2-O₃ and LINTULCC models versus an observed $9 \pm 11\%$. The enhancements
12 due to CO₂ were predicted to be $24 \pm 9\%$ and $42 \pm 11\%$, respectively, which straddled the
13 observed $30 \pm 22\%$ gain. Based on the 13 experiments that included all four treatments (\pm O₃,
14 \pm CO₂), an actual 10% yield loss due to O₃ at ambient CO₂ levels was reduced to a 4% loss by the
15 elevated CO₂. The AFRCWHEAT2-O₃ model predicted 7 and 4% losses, and LINTULCC
16 model predicted 8 and 5% losses due to the O₃ and O₃ + CO₂ treatments. The actual and
17 simulated yield increases in response to CO₂ increased further with increasing temperature, but
18 although temperature had no discernible effect on the observed depression of yield caused by O₃
19 alone, both models suggested that the yield reduction was diminished both by higher
20 temperatures and higher CO₂ levels.

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

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

23 where Y = grain yield, $\text{g} \cdot \text{m}^{-2}$; $[\text{CO}_2]$ = ppm CO₂; $[\text{O}_3]$ = ppb O₃, 12-h mean; $[T]$ = °C; $[I]$ = light
24 intensity, $\text{MJ} \cdot \text{m}^{-2}/\text{day}$; and $[\text{H}_2\text{O}]$ is a dummy variable: well watered = 1; limited water
25 supply = 2. (***, $p < 0.001$; **, $p < 0.01$; the coefficient for I was not significant). With $R^2 =$
26 0.3983, adjusted for 258 degrees of freedom, a large part of the variability was still unaccounted
27 for by the five variables. However, this analysis suggests that CO₂, O₃, temperature, and water-
28

1 status are important codeterminants of wheat yield, but assumes no interactions. Substitution in
2 the model at summer light intensities and with well-watered plants indicates that, at 20° C,
3 a doubling of CO₂ levels to 700 ppm alone would lead to a 29.5% increase in yield, while
4 50 ppb O₃ alone would decrease yield by 10.9%. With both gases at those levels, the yield
5 would only increase 20%, but with a concurrent temperature rise of 2 °C, it would shrink to a
6 9.6% increase.

7 Both studies, therefore, indicate an amelioration of the effects of O₃ by CO₂, the magnitude
8 of which would be reduced at warmer temperatures. However, they relate to a single crop whose
9 response to CO₂ is temperature-sensitive. Information about other species in which the effects
10 of CO₂ and temperature are additive are limited. However, Wolf and Van Oijen (2003) recently
11 described a model (LPOTCO) simulating the effects of changes in climatic variables, CO₂
12 and O₃ on tuber yield potential of irrigated potato (*S. tuberosum* cv. Bintje) over locations within
13 the European Union ranging from Finland to Italy. They noted that although increased CO₂, O₃,
14 and light intensity were predominant controlling factors, increased temperature also influenced
15 potential yields substantially, with increases in northern latitudes (attributed to a longer growing
16 season) but decreases in southern latitudes (attributed to decreased assimilate production).

17 A clear understanding of the complex interactions of increased CO₂ and temperature
18 with O₃ must await further experimentation or simulations. However, it seems likely that any
19 CO₂-induced amelioration of the adverse effects of O₃ on aspects of growth other than seed or
20 grain yield may be lessened or increased by increased temperature, depending upon the
21 temperature optima for the species, along the lines suggested by Rowland-Bamford (2000).

22 Other crop simulation models which incorporate O₃ and some of the various environmental
23 factors, including elements of climate change, have been reviewed by Kickert et al. (1999) and
24 Rötter and Van De Geijn (1999). However, to date, the applications tend to have focused on
25 interactions of O₃ with factors such as soil moisture or nutrient availability.

26 With forest trees, the situation has the added complexity of a perennial growth form and
27 the inevitability, over time, of subjection to additional environmental stresses such as nutrient-
28 limitation. Here, too, although numerous models of tree growth have been described, there
29 appear to have been few applications to interactions of O₃ and factors of climate change.
30 Constable et al. (1996) used TREGRO to model the growth of Ponderosa pine exposed to
31 three O₃ levels (0.5×, 1.0×, and 2× ambient), two levels of CO₂ (ambient and ambient +

1 200 ppm CO₂), and two temperature regimes (ambient and ambient + 4 °C). Plant growth was
2 predicted to be decreased 1, 19, and 39% by the three levels of O₃, respectively. Increased CO₂
3 reduced the loss at the highest O₃ level to 7%; however, the combination of elevated CO₂ with
4 the higher temperature more than overcame the adverse effects of O₃, leading to a 4% increase,
5 largely attributed to increased fine root mass. The authors suggested that, in relation to the
6 baseline conditions used in the simulations (Corvallis, OR), higher concentrations of CO₂ and O₃
7 and a warmer climate will have little impact on total-tree growth, but they noted the importance
8 of undertaking multiple stress studies in order to be able to make accurate forecasts of the impact
9 of such changes on forest trees.

10 More recently, Constable and Friend (2000) compared the capabilities of six published
11 process-based models (CARBON, ECOPHYS, PGSM, TRE-BGC, TREGRO, and W91) for
12 simulating tree response to elevated CO₂, O₃, and temperature. They concluded that, although
13 these models were capable integrators of the effects of various environmental factors on
14 individual processes such as photosynthesis, they were less reliable when extrapolating to
15 growth.

16 Although most of the research emphasis has been on simple CO₂ × O₃ interactions, a few
17 isolated studies of interactions have involved O₃, CO₂, and biotic environmental factors. Heagle
18 et al. (1994) observed that both O₃ and CO₂ tended to be additive in encouraging the growth of
19 spider mite (*Tetranychus urticae*) populations on clover. Infection of wheat with leaf rust
20 (*Puccinia recondita*) sensitized the plants to O₃ injury, but its severity was significantly reduced
21 in elevated CO₂ (Tiedemann and Firsching, 2000). The effects of O₃ and CO₂ on mycorrhizal
22 symbioses was studied by Kytöviita et al. (1999) who found that CO₂ did not ameliorate the
23 adverse effects of O₃ on the root growth of Aleppo pine and European white birch. In another
24 study with Aleppo pine, Kytöviita et al. (2001) noted that both O₃ and elevated CO₂ reduced
25 mycorrhiza-induced N-uptake by the roots. In Scots pine, Kasurinen et al. (1999) observed
26 transient effects of elevated CO₂ and O₃ on root symbiosis, but none of the effects persisted over
27 the 3 years of the study.

28 The soil water × O₃ × CO₂ interaction was experimentally investigated by Broadmeadow
29 and Jackson (2000) in Durmast oak, European ash, and Scots pine. No interactions were noted
30 with ash and pine; but with oak, elevated CO₂ ameliorated and irrigation exacerbated the effects
31 of O₃, although the resultant effects were essentially additive.

1 Booker (2000) noted that soil nitrogen levels interacted only slightly with O₃ and CO₂ in
2 determining the composition of cotton leaves and roots. Carbon dioxide reversed the inhibition
3 of leaf growth caused by O₃, but increased N-fertility tended to reduce this reversal.

4 Because of the small number of studies of possibly significant interactions of three or more
5 environmental factors, it is impossible to draw any sweeping conclusions as to how O₃, in the
6 context of global climate change, may affect relationships among plants and insects, diseases,
7 and symbionts or among plants and nutrients or other air pollutants. The only interaction that
8 has some degree of general support is the amelioration of adverse O₃ effects by elevated CO₂.

9 10 **AX9.3.8.2 Ozone-UV-B Interactions**

11 As noted in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996), depletion
12 of stratospheric O₃ by halofluorocarbons has resulted in increased intensities of UV-B radiation
13 (280 to 320 nm wavelengths) at the Earth's surface. The situation is discussed more fully in
14 Chapter 10.

15 While stratospheric O₃ depletion may result in increased surface UV-B irradiation,
16 absorption of UV-B is a property of the O₃ molecule regardless of its location; surface UV-B
17 flux is, therefore, also reduced by O₃ in the troposphere. Although only about 10% of the total
18 atmospheric O₃ column occurs in the troposphere (Fishman et al., 1990), it contributes a
19 disproportionately greater absorption effect than stratospheric O₃, because the UV radiation
20 penetrating the troposphere becomes increasingly diffuse as it reaches the surface, with a
21 consequent increase in mean path length (Brühl and Crutzen, 1989). Any benefits to vegetation
22 from reduced ambient O₃ stress must, therefore, also be viewed in the context of possible
23 adverse effects due to increased UV-B irradiation. There are, thus, two distinct types of possible
24 interactions between surface level O₃ and UV-B radiation:

- 25 • direct interactions involving simultaneous, sequential, or mixed exposures to O₃ and
UV-B stresses; and
- 26 • effects on responses to UV-B itself resulting from changes in radiation intensity caused
by changes in surface-level O₃ concentrations.

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

1 The most recent reviews specifically addressing the *combined* effects of tropospheric O₃
2 and UV-B on plants are by Runeckles and Krupa (1994) and Krupa and Jäger (1996), although
3 the topic has also been included in several more general reviews of O₃ effects and factors of
4 climate change, such as those by Unsworth and Hogsett (1996), Krupa et al. (1998), Posthumus
5 (1998), and Krupa and Groth (2000).

6 However, little new information has become available since Runeckles and Krupa (1994)
7 noted that the scanty knowledge of the effects of UV-B and O₃ combinations available at that
8 time was derived solely from studies of soybean. Miller et al. (1994) observed no interaction
9 and no effect of UV-B on yield, in contrast to a previous report by Teramura et al. (1990) using
10 the same cultivar, Essex. More recently, in a study of the saltmarsh grass *Elymus athericus*
11 subjected to reciprocal exposures to O₃ and UV-B, Van De Staaij et al. (1997) observed no
12 interactive effects and no adverse effects of UV-B following 14-day exposures, even though an
13 earlier report showed that longer exposures to UV-B (65 days) could cause a 35% loss of
14 biomass (Van De Staaij et al., 1993). However, in a study in which ambient, high-altitude UV-B
15 levels were compared with near-zero levels, at ambient or 2×-ambient levels of O₃, interactions
16 involving the levels of antioxidants in Norway spruce and Scots pine were reported by
17 Baumbusch et al. (1998). Schnitzler et al. (1999) subsequently reported that O₃-induced injury
18 and adverse effects on photosynthesis were more pronounced with near-zero UV-B levels,
19 indicating an amelioration of the O₃-response. A later study with Scots pine (Zinser et al., 2000)
20 revealed O₃ × UV-B interactions at the gene expression and biochemical levels. In contrast,
21 Ormrod et al. (1995) reported that UV-B predisposed *Arabidopsis thaliana* to injurious growth
22 effects from O₃ exposure.

23 At various organizational levels, Runeckles and Krupa (1994) identified several similarities
24 between plant response to O₃ and UV-B, and at the level of gene expression, there have recently
25 been several reports of both similarities and distinctions. Willekens et al. (1994) reported similar
26 effects of O₃, UV-B, and SO₂ on the expression of antioxidant genes in *Nicotiana*
27 *plumbaginifolia*. In parsley (*Petroselinum crispum*), Eckey-Kaltenbach et al. (1994a) found
28 that O₃ was a cross-inducer for both the UV-B-induced enhanced biosynthesis of flavonoids
29 and the pathogen-induced furanocoumarin phytoalexins, in keeping with the previously
30 observed O₃-induction of fungal and viral defense reactions. In this regard, Yalpani et al. (1994)

1 provided evidence that O₃ and UV-B acted similarly in increasing disease-resistance via a
2 salicylate-mediated enhancement of defense proteins in tobacco. However, subsequent work
3 with tobacco led Thalmair et al. (1996) to conclude that exposure to UV-B did not lead to the
4 accumulation of pathogenesis-related proteins. In Scots pine, although O₃ is known to induce
5 stilbene synthase and cinnamyl alcohol dehydrogenase, UV-B was found to enhance the former
6 but suppress the latter, revealing an interaction at the level of gene expression (Zinser et al.,
7 2000).

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

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

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

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

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

1 **AX9.3.9 Summary - Environmental Factors**

2 Although O₃ and other photochemical oxidants are phytotoxic, their actions on vegetation
3 may be modified by a host of biotic and abiotic factors in the environment; conversely, they may
4 modify plant response to these other factors. The extensive review of these biological, physical,
5 and chemical factors conducted for the 1996 O₃ AQCD (U.S. Environmental Protection Agency,
6 1996) concluded with a statement that our understanding was too fragmented to permit drawing
7 many general conclusions. With today's increased awareness of the need for more complete
8 information on interactions, it is unfortunate that, in the interval since the 1996 criteria
9 document, rigorous, systematic investigations of interactions have been rare, and most of the
10 new information is as fragmented as before. This is inevitable, partly in view of the vast scope
11 of the possible interactions between O₃ and other environmental variables and partly due to the
12 overall lack of funding for such research in these areas.

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

22 The situation is a little clearer with respect to interactions involving facultative
23 necrotrophic plant pathogens with O₃, generally leading to increased disease. With obligate
24 biotrophic fungal, bacterial, and nematode diseases, there are twice as many reports
25 indicating O₃-induced inhibitions than enhancements. The frequent reports that infection
26 by obligate biotrophs reduces the severity of O₃-induced foliar injury should not be interpreted
27 as "protection", because of the negative effects on the host plant of the disease per se. With
28 obligate biotrophs, the nature of any interaction with O₃ is probably dictated by the unique,
29 highly specific biochemical relationships between pathogen and host plant. At this time,
30 therefore, although some diseases may become more widespread or severe as a result of

1 exposure to O₃, it is still not possible to predict which diseases are likely to present the greatest
2 risks to crops and forests.

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

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

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

30 Although the relative humidity of the ambient air has generally been found to increase the
31 adverse effects of O₃ by increasing stomatal conductance and thereby increasing O₃ flux,

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

11 Mineral nutrients in the soil, other gaseous air pollutants, and agricultural chemicals
12 constitute chemical factors in the environment. The evidence regarding interactions with
13 specific nutrients is still contradictory. Some experimental evidence indicates that low general
14 fertility increases sensitivity to O₃, while simulation modeling of trees suggests that nutrient
15 deficiency and O₃ act less than additively; however there are too many example of contrary
16 trends to permit any sweeping conclusions. Somewhat analogously with temperature, it appears
17 that any shift away from the nutritional optimum may lead to greater sensitivity, but the shift
18 would have to be substantial before a significant effect on response to O₃ was observed.

19 Interactions of O₃ with other air pollutants have received relatively little recent attention.
20 The situation with SO₂ remains inconsistent, but seems unlikely to pose any additional risk to
21 those related to the individual pollutants. With NO and NO₂, the situation is complicated by
22 their nutritional value as N sources. In leguminous species, it appears that NO₂ may reduce the
23 impact of O₃ on growth, with the reverse in other species, but the nature of the exposure pattern,
24 i.e., sequential or concurrent, also determines the outcome. Much more investigation is needed
25 before we will be able to predict the outcomes of different O₃-NO-NO₂ scenarios. The latest
26 research into O₃ × acid rain interactions has confirmed that, at realistic acidities, significant
27 interactions are unlikely. A continuing lack of information precludes offering any
28 generalizations about interactive effects of O₃ with NH₃, HF, or heavy metals. More evidence
29 has been reported that the application of fungicides affords some protective effects against O₃.

30 Over the last decade, considerable emphasis has been placed on research into O₃
31 interactions with the components of global climate change: increased atmospheric CO₂,

1 increased mean global temperatures, and increased surface-level UV-B radiation. However,
2 most of these studies have tended to regard increased CO₂ levels and increased mean
3 temperatures as unrelated phenomena. Experiments into the effects of doubled CO₂ levels at
4 today's mean ambient temperatures are not particularly helpful in trying to assess the impact of
5 climate change on responses to O₃. To date, the limited experimental evidence and evidence
6 obtained by computer simulation suggest that in a 600+ ppm world, although the enriched CO₂
7 would more than offset the impact of O₃ on responses as varied as wheat yield or the growth of
8 young Ponderosa pine trees, the concurrent increase in temperature would reduce, but probably
9 not eliminate, the net gain. A similar decrease in the net gain resulting from the complete
10 reversal by CO₂ of the inhibition of photosynthesis caused by O₃ has been reported for increased
11 UV-B irradiation. However, these are preliminary results based on minimal data.

12 In conclusion, although the increased use of computer simulations may be important in
13 suggesting outcomes of the many complex interactions of O₃ and various combinations of
14 environmental factors, the results obtained will only be as reliable as the input data used for their
15 parameterization. The data needed for good simulations can only come from organized,
16 systematic study. For predicting the future, ignorance is as good as dependence on poor
17 simulations.

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6

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

2 **AX9.4.1 Introduction**

3 Indices are metrics that relate measured plant damage (i.e., reduced growth) to monitored
4 ambient O₃ concentration over time. An index is needed to provide a consistent metric for
5 reviewing and comparing exposure-response effects obtained from various studies. Such indices
6 may also provide a basis for developing a biologically-relevant air quality standard for protecting
7 ecological resources. The determination of O₃ impact on plants has been the effect on plant
8 growth and/or yield for purposes of the air quality standard setting process (U.S. Environmental
9 Protection Agency, 1986, 1996b). The quantifying function over some time frame has
10 frequently been referred to as “dose-response” and “exposure-response.” The distinction is in
11 how the pollutant concentration is measured: “dose” is the measured pollutant concentration
12 absorbed by the leaf over some time period, whereas “exposure” is the ambient air concentration
13 measured near the plant over some time period.

14 A measure of plant O₃ uptake from the ambient air (either rate of uptake or cumulative
15 seasonal uptake) is the ideal measure, because without O₃ or its reactive product(s) reaching the
16 target tissue there is no effect (Tingey and Taylor, 1982). Uptake is controlled in part by stomata
17 (see Section AX9.2 for a detailed discussion). An uptake measure should integrate all those
18 environmental factors that influence stomatal conductance, e.g., temperature, humidity, soil
19 water status. A direct measure of the internal leaf concentration of O₃, however, is technically
20 difficult and thus uptake values are generally obtained with simulation models that require
21 species- and site-specific variables. Because of this, a surrogate for uptake was sought early on
22 using statistical summaries of monitored ambient pollutant concentration over some integral of
23 time (Lee et al., 1988; Lefohn and Benedict, 1982; O’Gara, 1922; U.S. Environmental Protection
24 Agency, 1986, 1992, 1996b).

25 An index of exposure then must consider those abiotic and biotic factors known to modify
26 the plant response by altering O₃ uptake (Hogsett et al., 1988; U.S. Environmental Protection
27 Agency, 1996b), including the temporal dynamics of exposure (e.g., concentration, frequency,
28 duration), plant phenology (see Section AX9.3), plant defense mechanisms (e.g., antioxidants)
29 (see Section AX9.2), and site climate and soil factors (e.g., temperature, VPD, soil moisture)
30 (see Section AX9.3). The development of such indices continues to be a challenge (U.S.
31 Environmental Protection Agency, 1996b).

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

2 The 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996b) focused on the
3 research developing exposure indices to quantify growth and yield effects in crops, perennials,
4 and trees (primarily seedlings) and not foliar injury. The indices were various functional and
5 statistical summaries of monitored hourly O₃ concentrations over designated time periods. The
6 testing of the adequacy of these indices to order the measured responses of growth and/or yield
7 in crops and tree species as seedlings was accomplished through regression analyses of earlier
8 exposure studies. No direct experimental testing of the adequacy of these indices was
9 accomplished. Their development focused on consideration and inclusion of some, but not all,
10 factors that affect O₃ uptake and expression of effects (e.g., Lee et al., [1988]). The 1996
11 document (U.S. Environmental Protection Agency, 1996b) drew a number of conclusions that
12 built on even earlier conclusions (U.S. Environmental Protection Agency, 1992). Based on a
13 review of the research published since 1996, those conclusions are still valid.

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

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

30 Higher hourly averaged concentrations had a greater effect on plant response. It was
31 concluded that cumulative indices that gave greater weight to higher concentrations related well

1 with plant response (crops and tree seedlings) and ordered the treatment means in monotonically
2 decreasing fashion with increasing exposure, based on studies that applied two or more types of
3 exposure regimes with replicate studies of the same species. Examples of these indices, among
4 others, were: AOT60 (the seasonal sum of the difference between an hourly concentration above
5 the threshold value of 60 ppb, minus the threshold value of 60 ppb), SUM06 (the seasonal sum
6 of hourly concentrations at or above the threshold value of 60 ppb), W126 (a sigmoid functional
7 weighting of all hourly concentrations for the season), (U.S. Environmental Protection Agency,
8 1996b).

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

13 An exposure index that incorporated either the daily or seasonal temporal patterns of
14 higher concentration occurrence with the temporal pattern of individual species' stomatal
15 conductance was not reported in the 1996 O₃ AQCD (U.S. Environmental Protection Agency,
16 1996b). Based on available data, it was unresolved how to proceed with weighting time of day
17 or temporal patterns of species conductance.

18 The relative importance of cumulative peak concentrations (>0.10 ppm) versus cumulative
19 mid-range concentrations (0.05 to 0.099 ppm) was questioned. Although controlled experiments
20 had provided important evidence that the higher hourly average concentrations should be given
21 greater weight than the mid-level values in developing indices, there was concern that, under
22 ambient conditions in the field, the higher concentrations did not occur at the time of maximum
23 plant uptake. This coincidence was considered to be the critical factor in determining peak
24 concentration impacts on plants. Based on the evidence at that time, it was not possible to
25 conclude whether the cumulative effects of mid-range concentrations were of greater importance
26 than those of peak hourly average concentrations in determining plant response (U.S.
27 Environmental Protection Agency, 1996b). No direct experimental studies, however, had
28 addressed this question prior to 1996, nor have any since.

29 The composite exposure-response functions for crops and tree seedlings were derived from
30 single and multiyear exposure studies that used modified or simulated ambient exposure profiles.
31 These profiles were typified by episodic occurrence of a large number of higher O₃

1 concentrations. This type of pattern is not atypical but is not found in all rural agricultural and
2 some forested areas in the United States. Selecting a concentration value from these crop and
3 seedling response models may result in an over- or underestimation of growth effects if applied
4 to regions of the country where a different type of temporal pattern of occurrence is prevalent
5 (U.S. Environmental Protection Agency, 1996b). A multicomponent index was suggested that
6 combined the concentration-weighted, cumulative index with the number of occurrences of
7 hourly averaged concentrations ≥ 0.10 ppm that might reduce the uncertainty associated with
8 selecting the exposure value for protection based on NCLAN-type studies (Lefohn and Foley,
9 1992; Musselman et al., 1994; U.S. Environmental Protection Agency, 1996b). No direct
10 experimental studies addressed this question prior to 1996, nor have any since.

11 Since 1996, additional research has focused on the time of day when the higher hourly
12 average concentrations occur, the time of day of maximum plant uptake, the diurnal variability
13 of plant defense mechanisms, and various suggestions as to including these factors in any one of
14 the cumulative, concentration-weighted exposure indices. A much broader literature has focused
15 since 1996 on relating O₃ flux to plant response and how to use this as an index relating ambient
16 concentration to effects. These new developments are discussed in the sections that follow.

18 **AX9.4.3 Evaluation of Various Exposure Indices for Describing Ambient** 19 **Exposure-Response Relationships**

20 Mathematical approaches for summarizing ambient air quality information in biologically
21 meaningful forms that can serve as surrogates for dose for O₃ vegetation effects assessment
22 purposes have been explored for more than 80 years (O'Gara, 1922; U.S. Environmental
23 Protection Agency, 1996b). Several indices have attempted to incorporate some of the
24 biological, environmental, and exposure factors (directly or indirectly) that influence the
25 magnitude of the biological response and contribute to observed variability (Hogsett et al.,
26 1988). In the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996b), the exposure
27 indices were arranged into five categories; (1) One Event, (2) Mean, (3) Cumulative,
28 (4) Concentration Weighted, and (5) Multicomponent, and were discussed in detail (Lee et al.,
29 1989). Figure AX9-16 illustrates how several of the indices weighted concentration and
30 accumulate exposure.

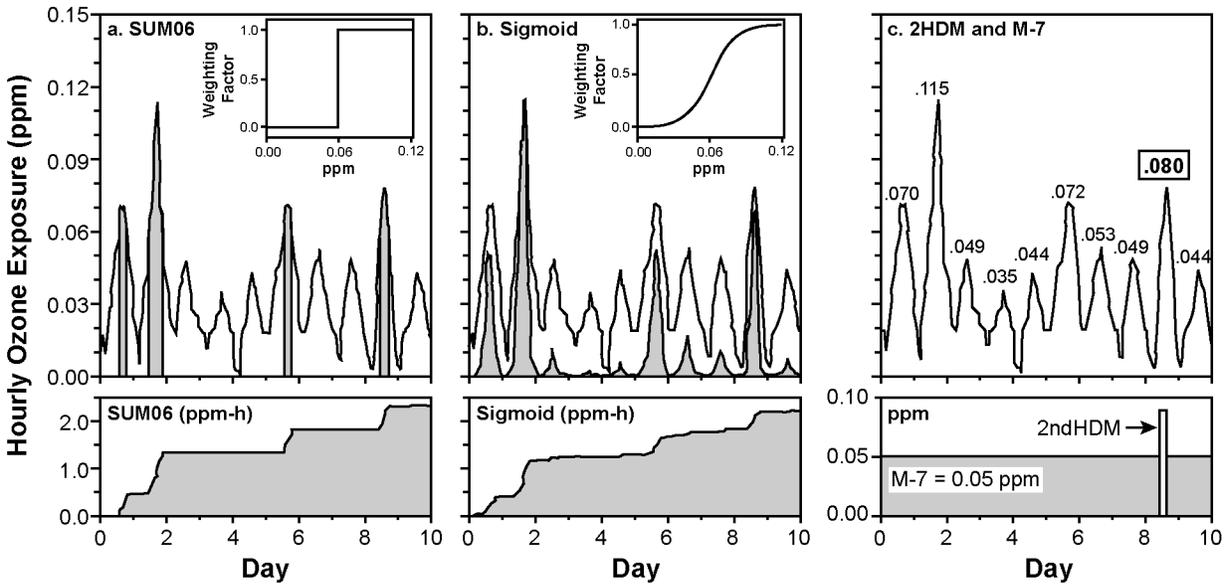


Figure AX9-16. Diagrammatic representation of several exposure indices, illustrating how they weight concentration and accumulate exposure. (a) SUM06: the upper graphic illustrates an episodic exposure profile; the shaded area under some of the peaks illustrates the concentrations greater than or equal to 0.06 ppm that are accumulated in the index. The insert shows the concentration weighting (0 or 1) function. The lower portion of the graphic illustrates how concentration is accumulated over the exposure period. **(b) SIGMOID:** the upper graphic illustrates an episodic exposure profile; the variable shaded area under the peaks illustrates the concentration-dependent weights that are accumulated in the index. The insert shows the sigmoid concentration weighting function. The midpoint of the sigmoid weighting scheme was 0.062 ppm. The lower portion of the graphic illustrates how concentration is accumulated over the exposure period. **(c) 2ndHDM and M-7:** the upper graphic illustrates an episodic exposure profile. The lower portion of the graphic illustrates that the 2ndHDM considers only a single exposure peak, while the mean applies a constant exposure value over the exposure period.

Source: Tingey et al. (1991).

1 Various components of the exposure-response relationship, including concentration, time
 2 of day, respite time, frequency of peak occurrence, plant phenology, predisposition, etc., were
 3 weighted with various functions and evaluated on their ability in ordering the regression of
 4 exposure versus growth or yield response. The statistical evaluations for each of these indices
 5 were accomplished using growth/yield response data from many earlier exposure studies (e.g.,

1 NCLAN). This retrospective approach was necessary, because there were no studies specifically
2 designed to test the goodness of fit of the various indices. The regression approach selected
3 those indices that most properly ordered and spaced the treatment means to optimize the fit of a
4 linear or curvilinear model. This approach provided evidence for the best indices, albeit not as
5 defensible as that from studies with experimental designs and analyses that focus on specific
6 components of exposure.

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

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

24 A statistical approach based on profile likelihoods was used to estimate parameters in
25 generalized exposure indices similar to the SUM06 and AOT40 indices (Blankenship and
26 Stefanski, 2001) using data from experiments conducted during 1993 at eight sites in the eastern
27 United States in which O₃-sensitive and -tolerant white clover genotypes were grown using
28 methods developed by Heagle et al. (Heagle et al., 1994). The results showed that for the
29 SUMX family of indices, where X is a cutoff value, hourly O₃ concentrations over ~71 ppb
30 contribute the most to yield prediction. For the AOTX family of indices, the parameter was
31 54.4. These values are similar to those used in the SUM06 and AOT40 indices already in use.

1 Furthermore, investigation of weighting for time of day confirmed the importance of the mid-
2 afternoon hours for this data set, unlike the results found for wheat in Sweden (Danielsson et al.,
3 2003; Pleijel et al., 2000a).

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

10 European scientists took a similar approach in developing indices describing growth and
11 yield loss in crops and tree seedlings, using OTCs with modified ambient exposures, but many
12 fewer species and study locations were employed in the European studies. There is evidence
13 from some European studies that a lower (Pleijel et al., 1997) or higher (Finnan et al., 1996,
14 1997) cutoff value may provide a better statistical fit to the experimental data. Finnan et al.
15 (1997) used seven exposure studies of spring wheat to confirm that cumulative exposure indices
16 emphasizing higher O₃ concentrations were best related to plant response and that cumulative
17 exposure indices using weighting functions, including cutoff concentrations, allometric and
18 sigmoidal, provided a better fit and that the ordering of these indices differed with different
19 linear or Weibull dose-response models. Weighting those concentrations associated with
20 sunshine hours in an attempt to incorporate a element of plant uptake did not improve the index
21 performance (Finnan et al., 1997). A more recent study using data from several European
22 studies of Norway spruce, analyzed the relationship between relative biomass accumulation and
23 several cumulative, weighted indices, including the AOT40 and the SUM06 (Skärby et al.,
24 2004). All the indices performed relatively well in regressing biomass and exposure index, with
25 the AOT20 and AOT30 doing slightly better ($r^2 = 0.46-0.47$). In another comparative study of
26 four independent data sets of potato yield and different cumulative uptake indices with different
27 cutoff values, a similarly narrow range of r^2 was observed (0.3 -0.4) between the different
28 cumulative uptake of O₃ indices (Pleijel et al., 2002).

29 In both the United States and Europe, the adequacy of these statistical summaries of
30 exposure in relating biomass and yield changes have, for the most part, all been evaluated using
31 data from studies not necessarily designed to compare one index to another (Lee et al., 1988,

1 1989; Skärby et al., 2004). But given the available data, the cumulative, concentration-weighted
2 indices perform better than the peak or mean indices. It is not yet possible, however, to
3 distinguish differences between the cumulative, concentration-weighted indices with direct
4 experimental studies.

5 The main conclusions from the 1996 O₃ AQCD (U.S. Environmental Protection Agency,
6 1996b) regarding an index based on ambient exposure are still valid. No information has come
7 forth in the interim to alter those conclusions significantly, and in fact, some recent studies have
8 further substantiated them. These key conclusions can be restated as follows:

- 9 • O₃ effects in plants are cumulative;
- 10 • higher O₃ concentrations appear to be more important than lower concentrations in
eliciting a response;
- 11 • plant sensitivity to O₃ varies with time of day and crop development stage;
- 12 • exposure indices that accumulate the O₃ hourly concentrations and preferentially weight
the higher concentrations have better statistical fits to growth/yield response than do the
mean and peak indices.

13 Following the 1996 criteria review process (U.S. Environmental Protection Agency,
14 1996a,b), the EPA proposed an alternative form of the secondary NAAQS for O₃ using a
15 cumulative, concentration-weighted exposure index to protect vegetation from damage (Federal
16 Register, 1997). The EPA considered three specific concentration-weighted indices: the cutoff
17 concentration weighted SUM06 and AOT60 and the sigmoid-weighted W126 exposure index
18 (U.S. Environmental Protection Agency, 1996a). All three indices performed equally well in
19 predicting the exposure-response relationships observed in the crop and tree seedlings studies
20 conducted during the previous 20 years (Lee et al., 1989). In a workshop convened to consider
21 the science supporting these indices, the participants agreed that all the cumulative
22 concentration- weighted indices considered were equally capable of predicting plant response
23 (Heck and Cowling, 1997).

24 The cutoff concentration-weighted index AOT40 was selected for use in Europe in
25 developing exposure-response relationships based on OTC studies of a limited number of crops
26 and trees (Grünhage and Jäger, 2003). The United Nations Economic Commission for Europe
27 (UNECE, 1988) adopted the critical levels approach for assessment of O₃ risk to vegetation
28 across Europe. As used by the UNECE, the critical levels are not air quality regulatory standards

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

27 A decision was made to work towards a flux-based approach for the critical levels
28 (“Level II”), with the goal of modeling O₃ flux-effect relationships for three vegetation types:
29 crops, forests, and seminatural vegetation (Grünhage and Jäger, 2003). Progress has been made
30 in modeling flux (see Section AX9.4.5 (Ashmore et al., 2004a,b) and the Mapping Manual is
31 being revised (Ashmore et al., 2004a,b; Grennfelt, 2004; Karlsson et al., 2003). The revisions

1 may include a flux-based approach for three crops: wheat, potatoes, and cotton. However,
2 because of a lack of flux-response data, a cumulative, cutoff concentration-based (AOTx)
3 exposure index will remain in use for the near future for most crops and for forests and
4 seminatural herbaceous vegetation (Ashmore et al., 2004a).

6 **AX9.4.4 Identifying Exposure Components That Relate to Vegetation Effects**

7 The efficacy of exposure indices in predicting biological responses requires that
8 researchers identify a relationship between measured growth and/or yield effects and exposure
9 components and those environmental and site factors that control pollutant uptake by the plant
10 effects. A number of these relationships were identified and discussed in the 1996 O₃ AQCD
11 (U.S. Environmental Protection Agency, 1996a). A significant, but in some instances,
12 unquantified role was identified for (1) concentration; (2) duration of exposure; (3) the diurnal
13 and seasonal patterns of exposure, e.g., time of day of peak event, season of higher exposures,
14 seasons of high precipitation and humidity, the frequency of occurrence of peak events to respire
15 time (peak to valley ratios); (4) plant phenology; (5) plant canopy structure; (6) meteorological
16 and site factors, e.g., light, humidity; and (7) plant defense mechanisms.

18 **AX9.4.4.1 Role of Concentration**

19 A significant role of higher concentrations was established earlier, based on several
20 experimental studies (U.S. Environmental Protection Agency, 1996b). Several studies since the
21 last review (Nussbaum et al., 1995; Oksanen and Holopainen, 2001; Yun and Laurence, 1999a)
22 have added support for the important role that peak concentrations, as well as the pattern of
23 occurrence, plays in plant response to O₃. Oksanen and Holopainen (2001) found that the peak
24 concentrations and the shape of the O₃ exposure (i.e., duration of the event) were important
25 determinants of foliar injury in European white birch saplings, but growth reductions were found
26 to be more related to total cumulative exposure. Based on air quality data from 10 U.S. cities,
27 three 4-week exposure treatments having the same SUM06 value were constructed by Yun and
28 Laurence (1999a). They used the regimes to explore effects of treatments with variable versus
29 uniform peak occurrence during the exposure period. The authors reported that the variable peak
30 exposures were important in causing injury, and that the different exposure treatments, although
31 having the same SUM06, resulted in very different patterns of foliar injury. Nussbaum et al.

1 (1995) also found peak concentrations and the pattern of occurrence to be critical in determining
2 the measured response. The authors recommended that to describe the effect on total forage
3 yield, peak concentrations >0.11 ppm must be emphasized by using an AOT with higher
4 threshold concentrations.

5 A greater role for higher concentrations affecting plant growth might be inferred based on
6 recent air quality analyses for the Southern California area (Lee et al., 2003; Tingey et al., 2004).
7 In the late 1960s and 1970s, extremely high O₃ concentrations had impacted the San Bernardino
8 NF. However, over the past 15 plus years, significant reductions in O₃ exposure have occurred
9 in the San Bernardino National Forest (Davidson, 1993; Lee et al., 2003; Lefohn and Shadwick,
10 2000; Lloyd et al., 1989). An illustration of this improvement in air quality is shown by the
11 37-year history of O₃ air quality at a site in the San Bernardino Mountains (Figure AX9-17)
12 (Lee et al., 2003). The O₃ exposure increased from 1963 to 1979 concurrent with increased
13 population and vehicular miles, followed by a decline to the present mirroring decreases in
14 precursor emissions. The pattern in exposure was evident in various exposure indices including
15 the cumulative concentration weighted (SUM06), as well as maximum peak event (1-h
16 peak), and the number of days having hourly averaged O₃ concentrations ≥95 ppb (i.e., the
17 California O₃ standard). The number of days having hourly averaged O₃ concentrations ≥95 ppb
18 declined significantly from 163 days in 1978 to 103 days in 1997. The changes in ambient O₃ air
19 quality for the site were reflected in the changes in the frequency and magnitude of the peak
20 hourly concentration and the duration of the exposure (Figure AX9-17). Considering the role of
21 exposure patterns in determining response, the seasonal and diurnal patterns in hourly O₃
22 concentration did not vary appreciably from year to year over the 37-year period (Lee et al.,
23 2003).

24 The inference for a role of higher concentrations comes both from results of ground
25 measures of tree conditions on established plots and from results of model simulations. Across a
26 broad area of the San Bernardino NF, the Forest Pest Management (FPM) method of injury
27 assessment indicated an improvement in crown condition from 1974 to 1988; and the area of
28 improvement in injury assessment is coincident with an improvement in O₃ air quality (Miller
29 and Rechel, 1999). A more recent analysis of forest changes in the San Bernardino NF using an
30 expanded network of monitoring sites has verified significant changes in growth, mortality rates,
31 basal area, and species composition throughout the area since 1974 (Arbaugh et al., 2003).

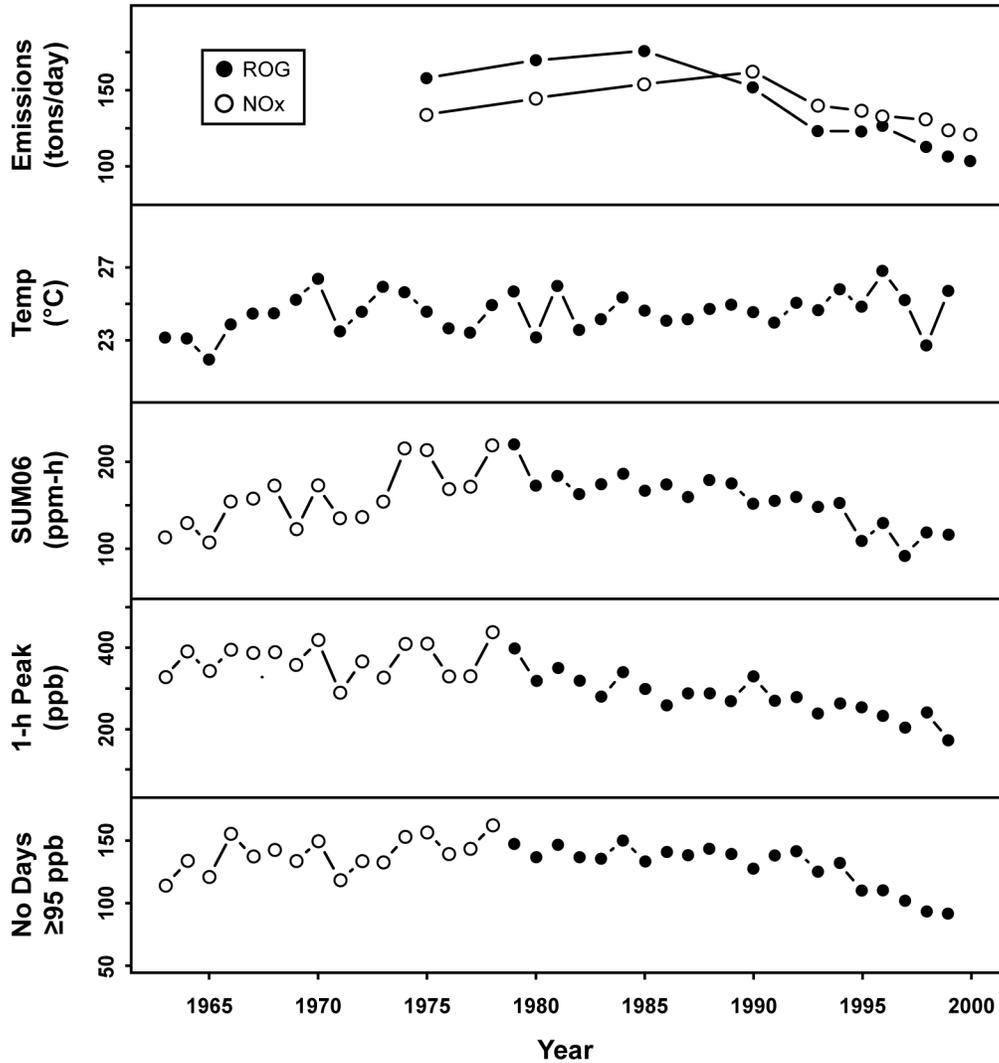


Figure AX9-17. Trends in May to September 12-h SUM06, peak 1-h O₃ concentration and number of daily exceedances of 95 ppb for Crestline in 1963 to 1999 in relation to trends in mean daily maximum temperature for Crestline and daily reactive organic gases (ROG) and oxides of nitrogen (NO_x) for San Bernardino county. Annual ROG and NO_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).

1 A model simulation of Ponderosa pine growth over the 40- year period in the San Bernardino NF
2 showed a significant impact of O₃ exposure on tree growth and indicates improved growth with
3 improving air quality. The improvement in growth was assigned to improved air quality, but no
4 distinction was made regarding the relative role of mid-range and higher hourly concentrations,
5 only that improved growth tracked decreasing SUM06, maximum peak concentration, and
6 number of days of hourly O₃ ≥95 ppb (Tingey et al., 2004). A summary of air quality data from
7 1980 to 2000 for the San Bernardino NF area of the number of “mid-range” hourly
8 concentrations indicated no dramatic changes over this 20-year period, ranging from about 1500
9 to 2000 hours per year (Figure AX9-18). There was a slow increase in the number of mid-range
10 concentrations from 1980 to 1986, which corresponds to the period after implementation of the
11 air quality standard. Another sharper increase was observed in the late 1990s. This pattern of
12 occurrence of mid-range hourly concentrations suggests a lesser role for these concentration
13 ranges compared to the higher values in either of the ground-level tree injury observations of the
14 model simulation of growth over the 40-year period.

16 **AX9.4.4.2 Role of Duration**

17 Recent studies have called into question the period of time over which concentrations are
18 accumulated and the form of the exposure index. Heagle and Stefanski (2000) reported that the
19 form of the exposure index was important only for 24-h indices for which SUM00 (cumulated all
20 hourly concentrations with no concentration weighting) provided the poorest fit. The authors
21 reported that the SUM00, SUM06, W95 (Lefohn and Runeckles, 1987), W126, and AOT40
22 produced similarly good fits of the foliage biomass data for 6-, 5-, and 4-h midday accumulating
23 periods. The study pooled data from San Bernardino (CA) and Riverside (CA) with data from
24 Amherst (MA), Corvallis (OR), Kennedy Space Center (FL), Raleigh (NC), and Blacksburg
25 (VA). Ozone exposures were much higher at the two California sites (indicated by high W126,
26 SUM06, W95, and AOT40 values) compared to the other locations. Because of the pooling of
27 the data, the large number of high hourly average O₃ concentrations that occurred at the
28 California sites may have resulted in the exposure indices being highly correlated with one
29 another and made it difficult to identify one optimal index.

30 In another study in California, Arbaugh et al. (1998) reported that the SUM00 exposure
31 index performed better for describing visible injury than the SUM06, W126, number of

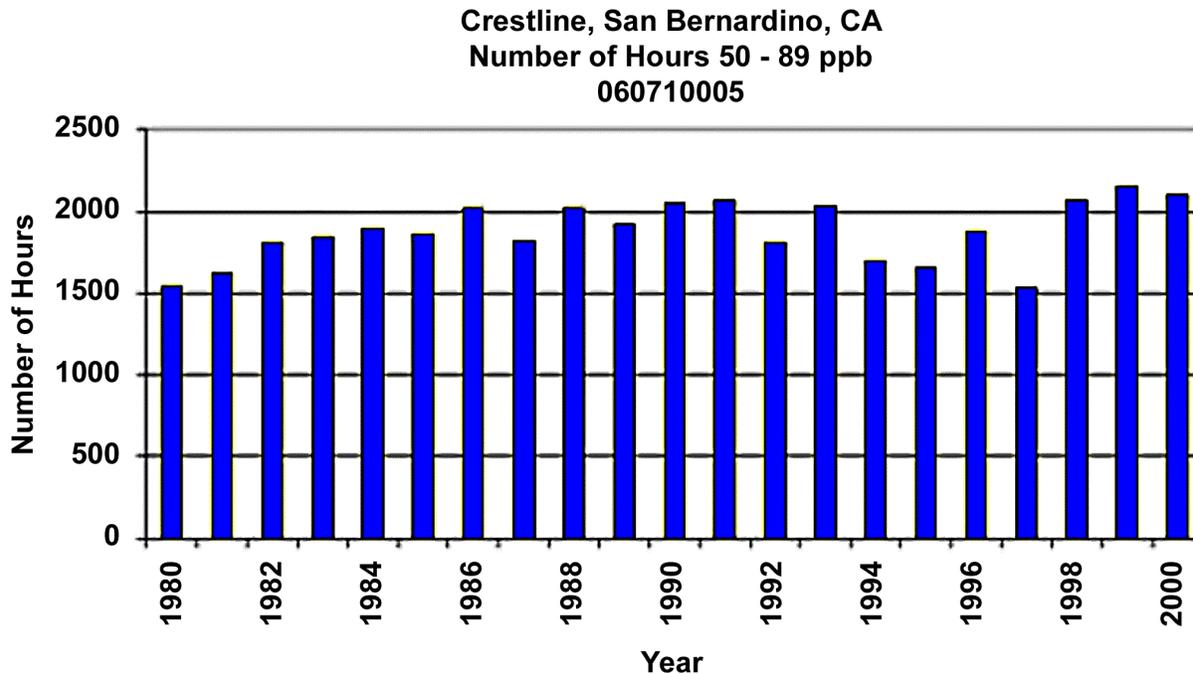


Figure AX9-18. The number of hourly average concentrations between 50 and 89 ppb for the period 1980 to 2000 for the Crestline, San Bernardino, CA monitoring site.

Source: U.S. Environmental Protection Agency (2003).

1 hours ≥ 0.08 ppm, and the number of days between measurement periods (U.S. Environmental
 2 Protection Agency, 1996b). These exposure indices were originally developed and tested using
 3 only growth/yield data, not foliar injury (U.S. Environmental Protection Agency, 1996b). This
 4 distinction is critical in comparing the efficacy of one index to another. However, for many
 5 locations in California, a large number of higher hourly average concentrations occur; thus the
 6 SUM00 could be highly correlated with the frequency of elevated hourly average concentrations
 7 and could be a good predictor of vegetation effects.

8

9 **AX9.4.4.3 Patterns of Exposure**

10 A significant factor in developing exposure indices is the temporal patterns of O₃
 11 occurrence over a day, a month, and a year, as well as seasonally overlaying the daily and
 12 seasonal temporal patterns of those influential climatic and site factors. The coincidence of

1 peak O₃ with maximal stomatal conductance and detoxification processes is key to affecting
2 plant growth response (Musselman and Minnick, 2000).

4 **Daily Patterns**

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

9 A 12-h daylight period for cumulating exposure was proposed following the 1996 O₃
10 AQCD based primarily on the assumption that most species probably do not have significant
11 conductance at night (U.S. Environmental Protection Agency, 1996a). An extensive review of
12 the literature, however, reported that a large number of species had varying degrees of nocturnal
13 stomatal conductance (Musselman and Minnick, 2000). The role of nighttime stomatal
14 conductance and O₃ exposure was demonstrated experimentally as well. Grulke et al. (2003)
15 showed that the stomatal conductance at night for Ponderosa pine in the San Bernardino NF
16 (CA) ranged from one tenth to one fourth that of maximum daytime gas exchange. In June, at
17 the high-elevation site, 11% of the total daily O₃ uptake of pole-sized trees occurred at night. In
18 late summer, however, O₃ uptake at night was negligible. Birch seedlings exposed to O₃ at night
19 show greater reductions in growth than those exposed to O₃ in daylight (Matyssek et al., 1995).
20 Field mustard (*Brassica rapa* L.) plants exposed to O₃ during the day or night showed little
21 significant difference in the amounts of injury or reduced growth response to O₃ treatment,
22 although the stomatal conductance was 70 to 80% lower at night (Winner et al., 1989). Tissue
23 biomass of Ponderosa pine seedlings was significantly reduced when seedlings were exposed to
24 either daytime or nighttime episodic profiles (Lee and Hogsett, 1999). However, the biomass
25 reductions were much greater with daytime peak concentrations than with nighttime peak
26 concentrations.

27 Although stomatal conductance was lower at night than during the day for most plants,
28 nocturnal conductance could result in some measurable O₃ flux into the plants. In addition,
29 plants might be more susceptible to O₃ exposure at night than during the daytime, because of
30 possibly lower plant defenses at night (Musselman and Minnick, 2000). Nocturnal O₃ flux also
31 depends on the level of turbulence that intermittently occurs at night. Massman (2004)

1 suggested that nocturnal stomatal O₃ uptake accounted for about 15% of the cumulative daily
2 effective O₃ dose that was related to predicted injury. Based on a review of the literature
3 regarding plant nocturnal stomatal conductance, Musselman and Minnick (2000) recommended
4 that any O₃ exposure index used to relate air quality to plant response should use the 24-h
5 cumulative exposure period for both exposure-response and effective flux models. However, in
6 an evaluation of a very large number of indices that described the O₃ impact on spring wheat,
7 Finnan et al. (1997) did not find any improvement in performance of the cumulative
8 concentration-weighted indices by weighting those concentrations occurring during sunlight
9 hours.

10 Stomatal conductances are species-dependent and linked to both diurnal and seasonal
11 meteorological activity as well as to soil/site conditions (e.g., soil moisture). Daily patterns of
12 leaf/needle conductance were often highest in midmorning, whereas higher ambient O₃
13 concentrations generally occurred in early to late afternoon when stomata were often partially
14 closed and conductances were lower. Total O₃ flux depends on atmospheric and boundary layer
15 resistances, both of which exhibit variability throughout the day. Recent experimental studies
16 with tree species demonstrated the decoupling of ambient O₃ exposure, peak occurrence, and gas
17 exchange, particularly in areas of drought (Panek, 2004). Several recent studies have suggested
18 that Ponderosa pine trees in the southern and northern Sierra Nevada Mountains may not be as
19 susceptible to high O₃ concentrations as to lower concentrations, due to reduced needle
20 conductance and O₃ uptake during the period when the highest concentrations occur (Arbaugh
21 et al., 1998; Bauer et al., 2000; Panek et al., 2002; Panek and Goldstein, 2001). Panek et al.
22 (2002) compared direct O₃ flux measurements into a canopy of Ponderosa pine and demonstrated
23 a lack of correlation of daily patterns of conductance and O₃ occurrence, especially in the late-
24 season drought period; they concluded that a consideration of climate or season was essential,
25 especially considering the role of soil moisture and conductance/uptake. In contrast, Grulke
26 et al. (2002) reported high conductance when O₃ concentrations were high in the same species,
27 but under different growing site conditions. The decoupling of conductance and higher ambient
28 O₃ concentration would hold true for more mesic environments as well as xeric landscapes. The
29 longer-term biological responses reported by Miller and Rechel (1999) for Ponderosa pine in the
30 same region, and the general reduction in recent years in ambient O₃ concentrations, suggest that

1 stomatal conductance alone may not be a sufficient indicator of potential vegetation injury or
2 damage.

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

7 8 **Seasonal Patterns**

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

24 Using TREGRO, a process-based tree growth model, Tingey et al. (2004) simulated long-
25 term growth of Ponderosa pine over a 37-year period. The simulation showed a high degree of
26 association between O₃ exposure and O₃-induced reductions in tree growth ($R^2 = 0.56$). The
27 scatter about the line, however, indicated that other factors beside O₃ are required to describe the
28 association between exposure and response. Incorporating annual temperature and precipitation
29 increased the R^2 to 0.67. In keeping with the observations of Panek (2004) on the decoupling of
30 peak O₃ occurrence and maximal conductance, the remaining unexplained variation is attributed
31 to differences in timing of peak O₃ uptake and peak O₃ exposure over the years.

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

2 Several earlier studies demonstrated the greater effect of episodic occurrence of O₃ peaks
3 compared to daily peak events (U.S. Environmental Protection Agency, 1996b). Since the
4 1996 O₃ AQCD, a few studies have corroborated the importance of this pattern in growth
5 response (Köllner and Krause, 2003; Yun and Laurence, 1999a; Nussbaum et al., 1995). Köllner
6 and Krause (2003) reported that, under equal exposure conditions, the most pronounced effects
7 on the yield of sugar beet (*Beta vulgaris* L.) and soybeans occurred with those regimes that
8 emphasized the episodic occurrence of peak events. Similarly, Yun and Laurence (1999b) used
9 exposure regimes constructed from 10 U.S. cities to demonstrate that variable peak occurrence
10 versus uniform occurrence was important in causing injury in tree seedlings. Nussbaum et al.
11 (1995) compared the effects of different patterns of peak occurrences with similar AOT40 values
12 and reported a stronger effect on total forage yield from the episodic treatment.

13 14 **AX9.4.4.5 Canopy Structure**

15 Another factor important in either O₃ exposure or uptake, is how canopy structure
16 affects O₃ concentration in and under forest canopies. There have been several investigations
17 of O₃ concentrations under tree canopies (Enders, 1992; Fontan et al., 1992; Fredericksen et al.,
18 1995; Joss and Graber, 1996; Kolb et al., 1997; Lorenzini and Nali, 1995; Neufeld et al., 1992;
19 Samuelson and Kelly, 1997). In general, they indicated a reduction in O₃ of ~20 to 40% in the
20 area below the canopy but above the shrub/herb layers. An essential component in the
21 determination of the AOT40 as a critical level was the height at which the O₃ concentration was
22 measured. The measurement heights are related to the O₃ concentration measured at the top of
23 the canopy, i.e., upper surface boundary of the (quasi-) laminar layer (Grünhage and Jäger,
24 2003). This location is presumably more related to stomatal uptake. Weighting the O₃
25 concentration at this location takes into account stomatal opening and, if weighted with the
26 Jarvis-Steward factors for radiation, temperature, and soil moisture, the “toxicologically”
27 effective AOT40 is obtained (Grünhage and Jäger, 2003). A question exists however as to
28 whether this “canopy” O₃ concentration is clearly connected to stomatal O₃ uptake. During site
29 conditions that limit stomatal conductance (e.g., low soil moisture, high VPD) at the top of the
30 canopy, high concentrations of O₃ can occur with minimal risk.

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

16 17 **AX9.4.4.6 Site and Climate Factors**

18 Soil moisture is a critical factor in controlling O₃ uptake through its effect on plant water
19 status and stomatal conductance. In an attempt to relate uptake, soil moisture, and ambient air
20 quality to identify areas of potential risk, available O₃ monitoring data for 1983 to 1990 were
21 used along with literature-based seedling exposure-response data from regions within the
22 southern Appalachian Mountains that might have experienced O₃ exposures sufficient to inhibit
23 growth (Lefohn et al., 1997). In a small number of areas within the region, O₃ exposures and
24 soil moisture availability were sufficient to possibly cause growth reductions in some O₃-
25 sensitive species (e.g., black cherry). The conclusions were limited, however, because of the
26 uncertainty in interpolating O₃ exposures in many of the areas and because the hydrologic index
27 used might not reflect actual water stress.

28 29 **AX9.4.4.7 Plant Defense Mechanism - Detoxification**

30 The non-stomatal component of plant defenses are the most difficult to quantify, but some
31 studies are available (Barnes et al., 2002; Chen et al., 1998; Massman and Grantz, 1995; Plöchl

1 et al., 2000), and a larger discussion can be found in Section AX9.3. Massman et al. (2000)
2 developed a conceptual model of a dose-based index to determine how plant injury response
3 to O₃ relates to the traditional exposure-based parameters. The index used time-varying-
4 weighted fluxes to account for the fact that flux was not necessarily correlated with plant injury
5 or damage. Their model applied only to plant foliar injury, and suggested that application of
6 flux-based models for determining plant damage (yield or biomass) would require a better
7 understanding and quantification of the injury and damage relationship.

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

10 Another approach in developing an index that relates growth response to ambient O₃ is
11 based on determining the O₃ concentration going from the atmosphere into the leaf, or flux.
12 Interest has been increasing in recent years, particularly in Europe, in using mathematically
13 tractable flux models for O₃ assessments at the regional and national scale (Emberson et al.,
14 2000a,b). Reducing uncertainties in flux estimates for areas with diverse surface or terrain
15 conditions to within ±50% requires “very careful application of dry deposition models, some
16 model development, and support by experimental observations” (Wesely and Hicks, 2000). As
17 an example, the annual average deposition velocity of O₃ among three nearby sites in similar
18 vegetation was found to vary by ±10%, presumably due to terrain (Brook et al., 1997).
19 Moreover, the authors stated that the actual variation was even greater, because stomatal uptake
20 was unrealistically assumed to be the same among all sites, and flux is strongly influenced by
21 stomatal conductance (Brook et al., 1997). This uptake-based approach to quantify the
22 vegetation impact of O₃ requires inclusion of those factors that control the diurnal and
23 seasonal O₃ flux to vegetation (e.g., climate patterns and species and/or vegetation-type factors
24 and site-specific factors). The models have to distinguish between stomatal and non-stomatal
25 components of O₃ deposition to adequately estimate actual concentration reaching the target
26 tissue of a plant to elicit a response. Determining this O₃ uptake via canopy and stomatal
27 conductance by necessity relies on models to predict flux and ultimately the “effective” flux
28 (Grünhage et al., 2004; Massman et al., 2000; Massman, 2004). “Effective flux” has been
29 defined as the balance between the O₃ flux and the detoxification process (Dämmgen et al.,
30 1993; Grünhage and Haenel, 1997; Musselman and Massman, 1999). The time-integrated
31 “effective flux” is termed “effective dose”. The uptake mechanisms and the resistances in this

1 process, including stomatal conductance and biochemical defense mechanisms, are discussed in
2 the previous Section AX9.3. The flux-based index is the goal for the “Level II” critical level for
3 assessment of ozone risk to vegetation and ecosystems across Europe (Ashmore et al., 2004a).

4 5 **AX9.4.5.1 Models of Stomatal Conductance**

6 Only a limited number of studies have measured O₃ concentration or its reaction products
7 within the leaf (e.g., Moldau and Bichele (2002); see Section AX9.4.3). Altimir et al. (2002)
8 described an enclosure technique for measuring O₃ flux to foliage at the shoot level that allowed
9 determination of partitioning and seasonality of the removal pathways on the foliage. The loss
10 of O₃ to the wall material of the chamber was great and required a correction when the stomatal
11 activity was low. Only a few instances of direct measures of O₃ flux to foliage in the field are
12 reported. Most measures of O₃ flux are from canopy measurements made with
13 micrometeorological techniques, but a number of assumptions are necessary and there are
14 limitations due to landscapes (Grünhage et al., 2000a; Wesely and Hicks, 2000). Comparison of
15 simulated and measured O₃ flux densities show good agreement in the mean (Grünhage et al.,
16 2000a). Comparison, however, of continuous O₃ concentrations and fluxes measured over a
17 5-year period by the gradient method (Fowler et al., 1989) in a 30-year-old Norway spruce stand
18 demonstrated a correlation over 5 years but were not correlated on a diurnal or seasonal basis.
19 The correlation was based on two uncoupled processes inside and outside the stomata, i.e., the
20 destruction of O₃ outside the stomata in the canopy was influenced by those same factors
21 (temperature, light, humidity) that control the diurnal opening and closing of the stomata.
22 A similar lack of correlation of measured concentration and estimated flux, daily and seasonally,
23 into Norway spruce and cembra pine (*Pinus cembra*) at six sites was due mostly to the control
24 of stomatal conductance by those same microenvironmental factors (temperature, humidity,
25 irradiance) (Emberson et al., 2000b). Seasonal variation of flux was attributed to the
26 temperature course. During the growing season, the leaf-air VPD was the environmental factor
27 controlling stomatal conductance and O₃ flux into the needles.

28 Given the limitations of actual measures of flux and the lack of correlation between
29 measured concentrations and flux, the effort is to develop an uptake or flux-based response index
30 using models that consider site, climatic, meteorological, and species-specific (e.g.,
31 detoxification reactions) factors. Models of O₃ conductance into plant tissue are available

1 (Grünhage et al., 1997; Massman, 1993; Wesely, 1989). The European Monitoring and
2 Evaluation Program (EMEP) developed an O₃ deposition model for application across Europe in
3 conjunction with the EMEP photochemical model as a tool for the critical levels program
4 (Emberson et al., 2000a). The model was developed to estimate vegetation type-specific O₃
5 deposition and stomatal flux, calculated according to a standard three-resistance formulation
6 incorporating atmospheric, boundary layer, and stomatal resistances (Emberson et al., 2000a).
7 The model used a multiplicative algorithm of the stomatal conductance of O₃ (Jarvis, 1976) and
8 has been parameterized for 10 European tree species, seven crop species, and one type of
9 seminatural vegetation. The model calculates conductance as a function of leaf phenology,
10 temperature, photosynthetic flux density (PFD), VPD, and soil moisture deficit (SMD). The
11 environmental variables are site-specific (or regionally-specific). The most important factors
12 limiting O₃ with this model were VPD, SMD, and phenology (Emberson et al., 2000a). These
13 factors demonstrate the critical linkage of high VPD and stomatal closure, which typically co-
14 occur with high O₃ concentrations.

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

23 The complexity of using flux as an index of O₃ exposure for growth response is shown in
24 field studies that measured O₃ flux into Norway spruce and cembran pine (Emberson et al.,
25 2000b). They demonstrated that stomatal conductance was the main limiting factor for O₃
26 uptake and showed the dependence of that measure on crown position, needle age, and altitude.
27 Consideration of the role of climate illustrates the importance of a flux measure. Pleijel et al.
28 (2000b) reported the improved relationship of yield in spring and winter wheat grown in OTCs
29 in many areas across Europe when it was related to the cumulative stomatal O₃ uptake during the
30 grain-filling period. Compared to the AOT40, the cumulative uptake index estimated larger

1 yield losses in the relatively humid parts of western and northern Europe, while smaller yield
2 loss was estimated for the dry summer climates in southern and central Europe.

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

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

18 Concern about the complexity of the stomatal models and the data needed to model O₃
19 uptake has led some researchers to offer modified accumulated exposure indices that consider
20 the meteorological factors controlling uptake (Gerosa et al., 2004; Karlsson et al., 2004a). In a
21 study of subterranean clover in Austria, Belgium, and southern Sweden, Karlsson et al. (2004a)
22 reported on the performance of a modified accumulated exposure over the threshold (mAOT)
23 which was based on solar radiation and VPD. This index improved the relationship for observed
24 visible injury. But when modeled uptake of O₃ was derived from a simple stomatal conductance
25 model considering solar radiation, VPD, and air temperature, this index gave an even greater
26 improvement in the relationship to visible injury than did the ambient exposure index of AOT40
27 (Karlsson et al., 2004a). The added value of the mAOT was worthwhile, as was its lower degree
28 of complexity and data requirements compared to simulating O₃ uptake with stomatal models.
29 Based on a study of O₃ fluxes over a barley (*Hordeum vulgare* L.) field in Italy, a similar
30 modified exposure index was reported and referred to as “effective exposure” (Gerosa et al.,
31 2004). Their approach was similar in its consideration of physiological aspects in conjunction

1 with monitored O₃ concentrations. It also addressed the shortcomings of the data needs for
2 modeled O₃ uptake.

3 Models that partition O₃ uptake into stomatal and non-stomatal components are also now
4 available and predict a significant non-stomatal component in calculating O₃ flux (Altimir et al.,
5 2004; Bassin et al., 2004; Mikkelsen et al., 2004; Nikolov and Zeller, 2003; Nussbaum et al.,
6 2003; Zeller and Nikolov, 2000). Altimir et al. (2004) compared the relative contributions of
7 stomatal and non-stomatal sinks at the shoot level for Scots pine. Using the EMEP model with a
8 revised parameterization for Scots pine, they demonstrated that a major removal of O₃ was due
9 to the non-stomatal component; when a non-stomatal term was introduced dependent on ambient
10 relative humidity, the non-stomatal contribution to the total conductance was about 50%. Zeller
11 and Nikolov (2000) demonstrated a large non-stomatal O₃ uptake (41% of the total annual flux)
12 in subalpine fir at a site in southern Wyoming using the biophysical model FORFLUX. In a
13 5-year study of measured O₃ flux to a Norway spruce canopy, Mikkelsen et al. (2004) showed
14 monthly patterns of non-stomatal and stomatal deposition as part of total deposition to the
15 canopy. Their study demonstrated that daily means of O₃ concentration and fluxes averaged
16 over 5 years correlated well, but the correlation was based on two different noncoupled
17 processes outside and inside the stomata. The destruction of O₃ in the canopy was influenced by
18 temperature, light, and humidity, and these same factors influence stomatal opening, e.g.,
19 midday and night closure. Consequently, the diurnal O₃ concentration and O₃ flux do not
20 correlate at all during the growing season. The study estimated yearly stomatal uptake to be a
21 minimum of 21% of total deposition (i.e., non-stomatal uptake was as high as 80% of total).
22 The stomatal uptake was highest May to August (30 to 33%) and lowest November to February
23 (4 to 9%).

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

25 If O₃ flux were used as the only metric to predict vegetation injury or damage, the
26 prediction might be overestimated, because of nonlinear relationships between O₃ and plant
27 response (Amiro et al., 1984; Amiro and Gillespie, 1985; Bennett, 1979, 1996b, 1986; U.S.
28 Environmental Protection Agency, 1978). The nonlinearity in the response surface suggests the
29 existence of a biochemical threshold. Musselman and Massman (1999) suggested that those
30 species having high amounts of detoxification potential might show less of a relationship
31

1 between O₃ stomatal uptake and plant response. More recently, nonlinear relationships
2 between O₃ flux and yield were shown for potato (Pleijel et al., 2002) and spring wheat
3 (Danielsson et al., 2003). The relationship between O₃ flux and potato yield led to the use of
4 an instantaneous flux threshold to overcome the nonlinear relationship (Pleijel et al., 2002).
5 However, the authors did not report a substantial improvement in the mathematical fitting of the
6 model when applying the threshold. Most of the flux was accumulated below 0.06 ppm.
7 However, Danielsson et al. (2003) showed an improved relationship between O₃ uptake and
8 yield of spring wheat using a threshold of 5 nmoles m⁻² sec⁻¹ (0.24 mg m⁻² sec⁻¹). These results
9 suggest not all O₃ entering the stomata contribute to a reduction in yield, which depends to some
10 degree on the amount of internal detoxification occurring for each particular species (see Section
11 AX9.2). The cellular detoxification reactions and repair processes which both detoxify oxidants
12 as well as play central role in the carbon economy of the plant are another level of resistance
13 to O₃ reaching the target tissue (see Section AX9.2). The magnitude of the response is
14 determined by the amount of the pollutant reaching the target site and the ability of the plant to
15 reestablish homeostatic equilibrium. Thus, one would expect to observe a decoupling of O₃
16 uptake with vegetation effects, which would manifest as a nonlinear relationship between O₃ flux
17 and injury or damage.

18 Additional factors for inclusion in flux-based models to predict vegetation effects would be
19 the defense and repair mechanisms. However, the fact that the defense and repair mechanisms
20 vary diurnally as well as seasonally may make it extremely difficult to apply a mathematically
21 determined threshold to instantaneous flux measurements to calculate cumulative flux. The
22 threshold models do not allow for the temporal (i.e., daily and seasonal) variability of defense
23 mechanisms. Specifically, the relationship between conductance, O₃ concentration, and
24 defense/repair mechanisms needs to be included. Recently, Massman (2004) illustrated that the
25 combination of stomatal conductance, O₃ concentration, and diurnal variation of defense
26 mechanisms showed the daily maximum potential for plant injury (based on effective dose)
27 coincided with the daily peak in O₃ mixing ratio. Massman et al. (2000) stressed that the product
28 of the overlapping mathematical relationships of conductance, concentration, and defense
29 mechanisms results in a much different picture of potential impact to vegetation than just the use
30 of conductance and concentration in predicting vegetation effects.

1 **AX9.4.5.3 Simulation Models**

2 Another approach for determining O₃ uptake and relating growth response to ambient O₃
3 exposure may be the use of physiologically-based simulation models. Several of these have
4 been used in various contexts, comparing O₃ response in a number of tree species with varying
5 climate and site factors (e.g., soil moisture) (Hogsett et al., 1996; Laurence et al., 2001; Ollinger
6 et al., 1997, 1998; Weinstein et al., 2001, 2002). These process-based models provide for an
7 integration of species, climate, and site factors controlling O₃ uptake with long-term growth.
8 One of the important considerations in applying simulation modeling is to carefully assess the
9 uncertainties associated with the modeling predictions. Further efforts need to be made to
10 exercise the models so that they predict past growth losses associated with changes in O₃
11 exposures that can be verified with on-the-ground surveys.

12 13 **AX9.4.6 Summary**

14 A large number of studies pertinent to the development of exposure indices have been
15 published since 1996, and these are predominantly focused on the development of a flux-based
16 index to relate ambient O₃ to effects. There were only a few such studies prior to 1996 and these
17 were reviewed in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996b). The few
18 studies published since 1996 on the role of O₃ exposure components (including concentration,
19 duration, and exposure patterns) in describing growth response to O₃ exposures have
20 substantiated earlier conclusions of the importance of higher concentration, shape of the peak,
21 and the episodicity of peak occurrence in the plant response to O₃ exposure. An inferred role of
22 peak concentrations is possible from consideration of improved air quality in regions such as the
23 San Bernardino Mountains in southern California. Studies provide the basis for focusing on the
24 higher O₃ concentrations, while including the lower levels, when estimating the effects of
25 emission reductions on vegetation.

26 A few studies have demonstrated the potential disconnection of the temporal patterns of
27 peak events and maximal stomatal conductance. In addition, a few other studies have
28 demonstrated the uptake of O₃ during nighttime hours, suggesting the need to cumulate O₃
29 exposure 24 h per day and not just during daylight hours.

30 Several studies since 1996 have demonstrated another critical concern in developing an
31 index for exposure. The concern is that peak O₃ events and maximum stomatal conductance

1 may be temporally separate. This disconnection introduces uncertainty in assessing O₃ impact
2 when using the current ambient exposure based cumulative, concentration-weighted indices.
3 If stomatal conductance is relatively low, as in the late afternoon in arid climates, and that is the
4 same time as the peak O₃ concentrations, then use of an exposure index that does not consider
5 this disconnect will overestimate the effect of the exposure. This concern is especially apparent
6 when assessing the impact of O₃ across all the varied climatic regions of the United States or
7 Europe. Some studies use stomatal models to predict uptake (Ashmore et al., 2004a) or
8 physiological process-based models (Laurence et al., 2001) to integrate those species, climate,
9 and site factors that drive this temporal pattern of stomatal conductance and exposure, and thus
10 reduce some of the uncertainty in regional and national assessments of effects. These
11 approaches, however, are still limited by being species-dependent.

12 The results of these studies and reviews indicate the need to continue to develop indices
13 that are more physiologically and meteorologically connected to the actual dose of O₃ the plant
14 receives. The cumulative concentration-weighted exposure indices are acknowledged surrogates
15 for effective dose and are simple conceptually and easy to measure. They do not fully
16 characterize the potential for plant uptake and resulting effects associated with O₃, because the
17 indices, being measures of ambient concentration, do not include the physical, biological, and
18 meteorological processes controlling the transfer of O₃ from the atmosphere through the leaf and
19 into the leaf interior (U.S. Environmental Protection Agency, 1996b). Use of such indices is
20 especially limited in spatial risk characterizations, because of the lack of linkage between
21 meteorology and species- and site-specific factors influencing O₃ uptake. The flux-based
22 approach should provide an opportunity to improve upon the concentration-based (i.e., exposure
23 indices) approach. A cautionary argument was advanced in a few publications centered around
24 the nonlinear relationship between O₃ uptake and plant injury (not growth alteration) response.
25 The concern was that not all O₃ stomatal uptake results in a reduction in yield, which depends to
26 some degree on the amount of internal detoxification occurring with each particular species;
27 species having high amounts of detoxification potential may show less of a relationship
28 between O₃ stomatal uptake and plant response.

29 The European approach and acceptance of flux-based critical values is a recognition of this
30 problem; a concerted research effort is needed to develop the necessary experimental data and

1 modeling tools that will provide the scientific basis for such critical levels for O₃ (Dämmgen
2 et al., 1994; Fuhrer et al., 1997; Grünhage et al., 2004).

3 At this time, based on the current state of knowledge, exposure indices that differentially
4 weight the higher hourly average O₃ concentrations but include the mid-level values represent
5 the best approach for relating vegetation effects to O₃ exposure in the United States. A large
6 database exists that has been used for establishing exposure-response relationships. Such a
7 database does not yet exist for relating O₃ flux to growth response. The pattern disconnects
8 between period of uptake and peak occurrence, as well as the potential for nocturnal uptake,
9 should be considered by adding some weighting functions into the currently used exposure
10 indices. Of particular consideration would be their inclusion in regional-to-national estimations
11 of O₃ impacts on vegetation. Another useful approach to regional assessment for certain species
12 is to simulate growth effects with process-based models that account for seasonal climate and
13 site factors that control conductance.

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

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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 O₃ 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 O₃ AQCDs dealt primarily with the effects of O₃ on agricultural crop species (U.S. Environmental Protection Agency, 1978, 1986). The chapter on vegetation effects in the 1978 O₃ 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 O₃ AQCD reviewed a substantial new body of evidence based on OTC experiments (see Section AX9.1) showing that ambient O₃ exposures reduced the growth and yield of herbaceous plants, again with a focus on major crop species. In the 1986 and 1996 O₃ 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 O₃ 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 O₃ exposures to produce response functions for estimating O₃ exposures that reduce growth by 10 or 30%. Also, in the 1986 and 1996 O₃ AQCDs, data from studies using EDU as a protectant were reviewed. The 1978, 1986, and 1996 O₃ AQCDs also reviewed data on the response to O₃ 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 O₃ 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

1 as damage, their occurrence indicates that phytotoxic concentrations of O₃ are present, and,
2 therefore, studies should be conducted to assess the risk to vegetation.

3 Visible symptoms due to O₃ exposures reduce the market value of certain crops and
4 ornamentals for which leaves are the product, e.g., spinach, petunia, geranium, and poinsettia.
5 The concept of limiting values used to summarize foliar symptoms in the 1978 O₃ AQCD (U.S.
6 Environmental Protection Agency, 1978) was also considered valid in the 1986 O₃ AQCD (U.S.
7 Environmental Protection Agency, 1986). Jacobson (1977) developed limiting values by
8 assessing the available scientific literature and identifying the lowest exposure
9 concentration/duration reported to cause foliar symptoms in a variety of plant species. Graphical
10 analyses presented in those documents indicated that the limit for reduced plant performance was
11 an exposure to 50 ppb for several hours per day for more than 16 days. Decreasing the exposure
12 period to 10 days increased the concentration required to cause symptoms to 100 ppb; and a
13 short, 6-day exposure further increased the concentration required to cause symptoms to
14 300 ppb. These limiting values established in 1978 were still deemed appropriate in the 1986
15 and 1996 O₃ AQCDs. Such foliar symptoms are caused by O₃ concentrations that occur in the
16 United States as shown in Table AX9-13 (adapted from U.S. Environmental Protection Agency,
17 (U.S. Environmental Protection Agency, 1996).

18 The 1986 O₃ AQCD emphasized that, although foliar symptoms on vegetation are often an
19 early and obvious manifestation of O₃ exposure, O₃ effects are not limited to foliar symptoms.
20 Other effects include reduced growth of many organs (including roots), changes in crop quality,
21 and alterations in plant susceptibility to biotic stressors and sensitivity to abiotic stressors. The
22 1986 O₃ AQCD also emphasized that O₃ exerts phytotoxic effects only if a sufficient amount
23 of O₃ reaches sensitive sites within the leaf (Section AX9.2). Ozone injury will not occur if the
24 rate of O₃ uptake is low enough that the plant can detoxify or metabolize O₃ or its metabolites or
25 if the plant is able to repair or compensate for the effects (Tingey and Taylor, 1982; U.S.
26 Environmental Protection Agency, 1986). Cellular disturbances that are not repaired or
27 compensated for are ultimately expressed as foliar symptoms, reduced root growth, or reduced
28 yield of fruits or seeds.

29 Beginning in the 1986 O₃ AQCD and continuing in the 1996 O₃ AQCD, OTC studies that
30 better quantified the relationship between O₃ exposure and effects on crop species were
31 reviewed, with a focus on yield loss. These studies can be grouped into two types, depending on

Table AX9-13. Summary of Ozone Exposure Indices Calculated for 3- or 5-Month Growing Seasons from 1982 to 1991^a

<i>3-Month Growing Season (June-August)</i>											
Year	No. of Sites ^b	HDM2 ^c ppm		M7 ppm		SUM00 ppm·h		SUM06 ppm·h		SIGMOID ppm·h	
		Mean	CV ^d	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1982	99	0.114	23.7%	0.05	18.7%	82.9	19.1%	26.8	68.8%	26.3	56.7%
1983	102	0.125	24.9%	0.06	21.9%	86.1	22.1%	34.5	58.1%	33.0	52.3%
1984	104	0.117	24.6%	0.05	18.2%	84.1	19.9%	27.7	58.4%	27.4	47.9%
1985	117	0.117	24.6%	0.05	17.1%	84.6	18.0%	27.4	59.6%	27.4	47.6%
1986	123	0.115	21.8%	0.05	19.1%	85.3	18.0%	27.7	65.0%	27.7	51.8%
1987	121	0.119	22.9%	0.06	17.6%	86.9	17.3%	31.2	56.4%	30.4	46.8%
1988	139	0.129	21.3%	0.06	17.8%	97.6	19.6%	45.2	46.8%	42.9	42.4%
1989	171	0.105	23.1%	0.05	17.5%	86.4	19.9%	24.8	78.7%	25.8	59.4%
1990	188	0.105	21.6%	0.05	18.3%	85.7	21.0%	25.8	76.2%	26.6	59.2%
1991	199	0.106	22.0%	0.05	18.4%	87.7	21.3%	28.3	74.2%	28.9	59.5%
<i>Among Years</i>		0.113	11.1%	0.05	10.0%	87.0	9.9%	29.5	42.1%	29.4	31.0%

<i>5-Month Growing Season (May-September)</i>									
Year	No. of Sites	M7 ppm		SUM00 ppm·h		SUM06 ppm·h		SIGMOID ppm·h	
		Mean	CV	Mean	CV	Mean	CV	Mean	CV
1982	88	0.048	20.6%	122.9	22.3%	37.3	70.9%	37.1	57.8%
1983	87	0.051	22.1%	129.6	24.4%	44.4	61.9%	43.8	52.7%
1984	95	0.048	18.0%	126.2	19.1%	36.7	60.8%	37.6	46.9%
1985	114	0.048	18.4%	124.5	19.4%	36.2	63.8%	37.0	50.3%
1986	118	0.048	20.3%	123.3	21.4%	34.9	70.7%	35.6	55.7%
1987	116	0.050	20.3%	128.7	20.4%	42.2	62.0%	41.8	50.3%
1988	134	0.054	18.7%	141.7	22.0%	58.0	50.5%	55.6	45.0%
1989	158	0.047	18.6%	127.8	22.5%	32.7	87.8%	35.2	64.1%
1990	172	0.049	19.8%	129.4	22.7%	34.6	82.7%	37.0	62.1%
1991	190	0.050	19.8%	130.6	23.6%	36.8	80.7%	38.8	62.9%
<i>Among Years</i>		0.049	9.8%	129.0	9.9%	38.7	42.5%	39.6	29.8%

^a 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.

^b 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.

^c 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.

^d CV = coefficient of variation.

Source: Table 5-30 from U.S. Environmental Protection Agency (1996) based on Tingey et al. (1991).

1 the experimental design and statistical methods used to analyze the data: (1) studies that
2 developed predictive equations relating O₃ exposure to plant response, and (2) studies that
3 compared the effects of discrete treatment level(s) to a control. The advantage of the regression
4 approach is that exposure-response models can be used to interpolate results between treatment
5 levels.

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

15 By the time the 1986 O₃ AQCD was prepared, much new information concerning the
16 effects of O₃ on the yield of crop plants had become available through EPA's NCLAN research
17 program and through research funded by other agencies. The NCLAN project was initiated by
18 the EPA in 1980 primarily to improve estimates of yield loss under field conditions and to
19 estimate the magnitude of crop losses caused by O₃ throughout the United States (Heck et al.,
20 1982; Heck et al., 1991). The cultural conditions used in the NCLAN studies approximated
21 typical agronomic practices. The primary objectives were:

- 22 (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;
- 23 (2) to assess the national economic consequences resulting from O₃ exposure of major
agricultural crops; and
- 24 (3) to advance understanding of cause-and-effect relationships that determine crop
responses to pollutant exposures.

25 Using NCLAN data, the O₃ concentrations predicted to cause 10 or 30% yield loss were
26 estimated using linear or Weibull response functions. The data in Table AX9-14 are from the
27 1996 document and were based on yield-response functions for 38 species or cultivars developed
28 from studies using OTCs of the type developed by Heagle et al. (1973) (see Section AX9.1).

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

<i>50th PERCENTILE^b</i>	SUM06	SE ^c	SIGMOID	SE	M7	SE	2HDM	SE
NCLAN Data (n = 49; wet and dry) ^d	24.4	3.4	21.5	2.0	0.049	0.003	0.094	0.006
NCLAN Data (n = 39; wet only)	22.3	1.0	19.4	2.3	0.046	0.003	0.090	0.010
NCLAN Data (n = 54; wet and dry) ^e	26.4	3.2	23.5	2.4	NA	NA	0.099	0.011
NCLAN Data (n = 42; wet only) ^e	23.4	3.1	22.9	4.7	NA	NA	0.089	0.008
NCLAN Data (n = 10; wet)	25.9	4.5	23.4	3.2	0.041	0.001	0.110	0.042
NCLAN Data (n = 10; dry)	45.7	23.3	40.6	0.1	0.059	0.014	0.119	0.017
Cotton Data (n = 5)	23.6	2.3	19.3	2.3	0.041	0.001	0.066	0.032
Soybean Data (n = 13)	26.2	5.4	22.6	3.6	0.044	0.005	0.085	0.013
Wheat Data (n = 6)	21.3	15.2	19.3	12.7	0.061	0.018	0.098	0.059
Cotton Data (n = 5) ^e	30.0	12.7	27.2	12.8	NA	NA	0.075	0.012
Soybean Data (n = 15) ^e	23.9	6.5	22.0	8.0	NA	NA	0.088	0.008
Wheat Data (n = 7) ^e	25.9	10.5	21.4	9.4	NA	NA	0.097	0.028
<i>75th PERCENTILE^b</i>								
NCLAN Data (n = 49; wet and dry)	14.2	4.2	11.9	5.6	0.040	0.007	0.051	0.010
NCLAN Data (n = 39; wet only)	14.3	2.7	12.6	2.3	0.039	0.005	0.056	0.006
NCLAN Data (n = 54; wet and dry) ^e	16.5	4.3	14.5	3.2	NA	NA	0.073	0.006
NCLAN Data (n = 42; wet only) ^e	17.2	3.0	14.7	2.4	NA	NA	0.070	0.006
NCLAN Data (n = 10; wet)	16.4	3.7	13.7	3.2	0.040	0.001	0.080	0.032
NCLAN Data (n = 10; dry)	24.0	0.8	22.3	0.1	0.053	0.022	0.093	0.003
Cotton Data (n = 5)	21.8	5.0	17.5	2.8	0.041	0.001	0.065	0.014
Soybean Data (n = 13)	14.2	0.1	12.4	0.1	0.041	0.006	0.069	0.004
Wheat Data (n = 6)	11.7	2.5	10.9	2.4	0.054	0.032	0.062	0.035
Cotton Data (n = 5) ^e	21.1	6.0	16.7	5.7	NA	NA	0.070	0.034
Soybean Data (n = 15) ^e	15.3	4.1	13.4	4.1	NA	NA	0.078	0.007
Wheat Data (n = 7) ^e	5.1	2.6	8.5	3.4	NA	NA	0.054	0.027

^aSee Appendix A for abbreviations and acronyms.

^bThe numbers in parentheses are the number of cases used in deriving the various exposure levels.

^cStandard error (SE).

^dNCLAN 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.

^e24-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).

Source: U.S. Environmental Protection Agency (1996) modified from Tingey et al. (1991).

1 Composite exposure-response functions for both crops and tree seedlings as a function of O₃
2 exposure expressed as SUM06 are shown in Figure AX9-19. Review of these data indicate that
3 10% yield reductions could be predicted for more than 50% of experimental cases when:
4 (1) 12-h SUM06 values exceeded 24.4 ppm·h, (2) SIGMOID values exceeded 21.5 ppm·h, or
5 (3) 7-h seasonal mean concentrations were 50 ppb. The SIGMOID index is very similar to the
6 W126 index (see Section AX9.4 for further information about O₃ indices). Much lower values
7 are required for each index to protect 75% of experimental cases (Table AX9-14). Grain crops
8 were generally found to be less sensitive than other crops. The data summarized in the 1996
9 criteria document also indicated that the variation in sensitivity within species may be as great as
10 differences between species.

11 The chemical protectant, EDU, was also used to provide estimates of yield loss. The
12 impact of O₃ on yield was determined by comparing the yield data from plots treated with EDU
13 versus untreated plots. Studies indicated that yields were reduced by 18 to 41% when daytime
14 ambient O₃ concentrations exceeded 80 ppb for 5 to 18 days over the growing season. For this
15 approach to be credible, the effects of EDU itself on a particular species must be preestablished
16 under conditions without O₃ exposure (Kostka-Rick and Manning, 1992).

17 The 1996 O₃ AQCD reviewed several experiments demonstrating that the seedlings of
18 some tree species such as poplars (*Populus* spp.) and black cherry are as sensitive to O₃ as are
19 annual plants, in spite of the fact that trees are longer-lived and generally have lower rates of gas
20 exchange, and, therefore, a lower uptake of O₃. The 1996 document also reviewed data showing
21 that O₃ exposures that occur at present in the United States are sufficient to affect the growth of a
22 number of trees species. For example, exposure-response functions for 51 cases of tree seedling
23 responses to O₃, including 11 species representing deciduous and evergreen growth habits,
24 suggest that a SUM06 exposure for 5 months of 31.5 ppm·h would protect hardwoods from a
25 10% growth loss in 50% of the cases studied (Table AX9-15). Similarly, a SUM06 exposure of
26 42.6 ppm·h should provide the same level of protection for evergreen seedlings. However, these
27 results do not take into the account the possibility of effects on growth in subsequent years.
28 Because multiple-year exposures may cause a cumulative effect on the growth of some trees
29 (Simini et al., 1992; Temple et al., 1992), it is likely that a number of species are currently being
30 affected even at ambient exposures (Tables AX9-13, AX9-21).

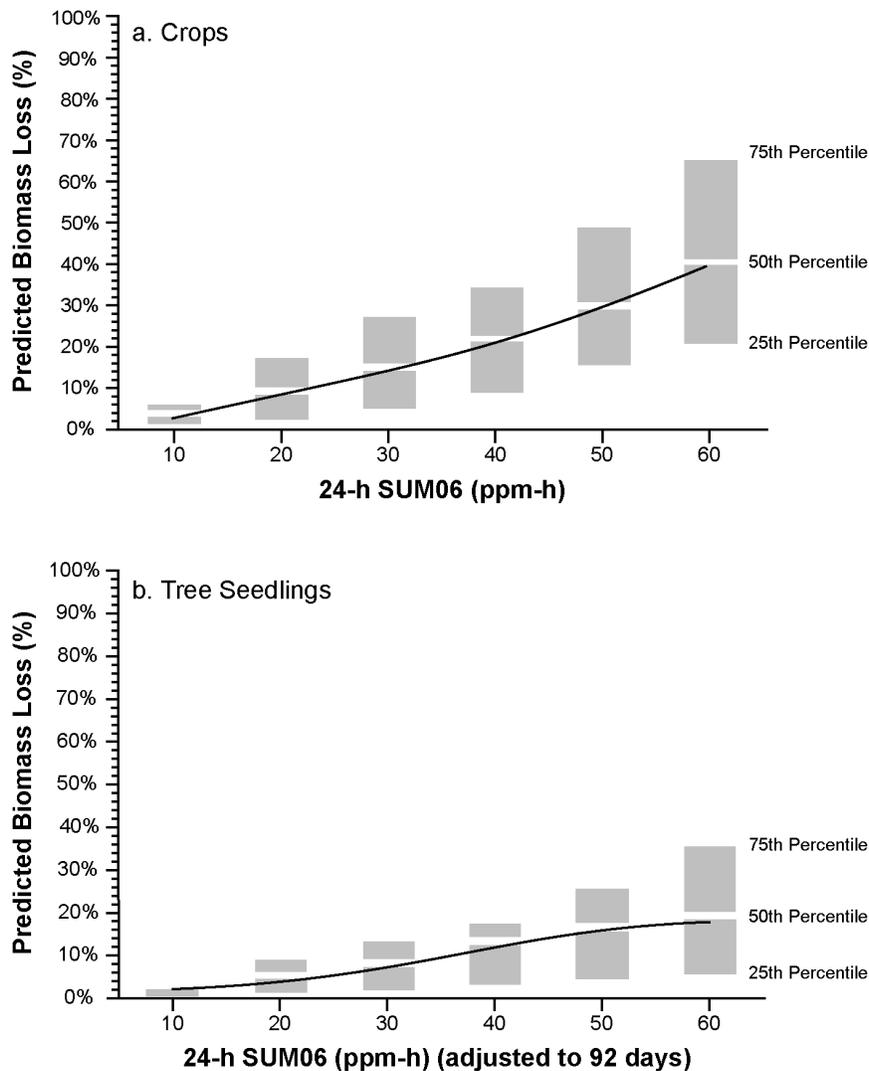


Figure AX9-19. Distribution of biomass loss predictions from Weibull and linear exposure-response models that relate biomass to O₃ exposure. Exposure is characterized with the 24-h SUM06 statistic using data from (a) 31 crop studies from National Crop Loss Assessment Network (NCLAN) and (b) 26 tree seedling 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.)^a**

Weibull Equations (all 51 seedling studies):

50th Percentile PRYL^b = 1 - exp(-[SUM06/176.342]**1.34962)

75th Percentile PRYL = 1 - exp(-[SUM06/104.281]**1.46719)

Weibull Equations (27 fast-growing seedling studies):

50th Percentile PRYL = 1 - exp(-[SUM06/150.636]**1.43220)

75th Percentile PRYL = 1 - exp(-[SUM06/89.983]**1.49261)

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

50th Percentile PRYL = 1 - exp(-[SUM06/190.900]**1.49986)

75th Percentile PRYL = 1 - exp(-[SUM06/172.443]**1.14634)

Weibull Equations (28 deciduous seedling studies):

50th Percentile PRYL = 1 - exp(-[SUM06/142.709]**1.48845)

75th Percentile PRYL = 1 - exp(-[SUM06/87.724]**1.53324)

Weibull Equations (23 evergreen seedling studies):

50th Percentile PRYL = 1 - exp(-[SUM06/262.911]**1.23673)

75th Percentile PRYL = 1 - exp(-[SUM06/201.372]**1.01470)

Levels Associated with Prevention of a 10 and 20% Total Biomass Loss for 50 and 75% of the Seedlings

All 51 Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	33.3	22.5
Biomass Loss	20%	58.0	37.5

27 Fast-Growing Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	31.3	19.4
Biomass Loss	20%	52.9	32.4

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.)^a

24 Slow-to-Moderate-Growth Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	42.6	24.2
Biomass Loss	20%	70.2	46.6

28 Deciduous Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	31.5	20.2
Biomass Loss	20%	52.1	33

23 Evergreen Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	42.6	21.9
Biomass Loss	20%	78.2	45.9

^aSee Appendix [XXX] for abbreviations and acronyms.

^bPRYL = predicted relative yield (biomass) loss

Source: U.S. Environmental Protection Agency (1996), based on Hogsett et al. (1995).

1 In 1986, the EPA (U.S. Environmental Protection Agency, 1986) established that 7-h per
 2 day growing season mean exposures to O₃ concentrations above 50 ppb were likely to cause
 3 measurable yield loss in agricultural crops. At that time, few conclusions could be drawn about
 4 the response of deciduous or evergreen trees or shrubs, due to the lack of information about
 5 response of such plants to season-long exposures to O₃ concentrations of 40 to 60 ppb and above.
 6 However, the 1978 and 1986 O₃ AQCDs (U.S. Environmental Protection Agency, 1978, 1986)
 7 indicated that the limiting value for foliar symptoms on trees and shrubs was 60 to 100 ppb for
 8 4 h. From 1986 to 1996, extensive research was conducted, establishing the sensitivity of many
 9 tree species. Based on research published since the 1986 O₃ AQCD (U.S. Environmental

1 Protection Agency, 1986), a number of conclusions were drawn in 1996 O₃ AQCD (U.S.
2 Environmental Protection Agency, 1996):

- 3 (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 O₃ 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).
- 4 (2) The results of OTC studies that compared yields at ambient O₃ exposures with
those in filtered air and retrospective analyses of crop data (Table AX9-14)
established that ambient O₃ 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 O₃ 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 O₃ concentrations observed in
the United States.
- 5 (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 O₃ 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).
- 6 (4) Depending on duration, concentrations of O₃ 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 O₃ exposures
(Tables AX9-13, AX9-21).
- 7 (5) Exposure-response functions for 51 cases of seedling response to O₃ (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.
- 8 (6) Studies of the response of trees to O₃ have established that, in some cases (for
instance, poplars and black cherry), trees are as sensitive to O₃ as are annual
plants, in spite of the fact that trees are longer-lived and generally have lower gas
exchange rates, and, therefore, lower O₃ uptake.

- 1 (7) Use of the chemical protectant, EDU, is of value in estimating O₃-related losses in
2 crop yield and tree growth, provided that care is exercised in establishing
3 appropriate EDU dosages to protect the plants without affecting growth.

4 The major question to be addressed in the remainder of this section is whether new
5 information supports or alters the 1996 criteria document conclusions summarized above.
6 In particular, this section evaluates whether the response of plants to experimental treatments at
7 or near O₃ concentrations characteristic of ambient levels in many areas of the United States
8 (Tables AX9-13, AX9-21) can be compared to a control or reduced O₃ treatment to establish a
9 potential adverse effect. Before evaluating new information from the literature on O₃ effects on
10 vegetation, O₃ exposure indices used in O₃ studies and trends in O₃ exposure patterns during the
11 past two decades are briefly reviewed.

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

13 As recognized in both the 1986 and the 1996 criteria documents, the characterization and
14 representation of the exposure of vegetation to O₃ is problematic, because the specific aspects of
15 pollutant exposure that cause injury or damage are difficult to quantify. This issue was
16 addressed in Section AX9.4, and only a few points will be discussed here in order to provide a
17 context for interpreting data on exposure-response relationships. The most important effects
18 of O₃ on vegetation occur due to uptake of O₃ through stomata, with subsequent oxidative injury
19 that appears to be rather nonspecific (Section AX9.2). As has been discussed by numerous
20 authors during the last three decades, from a toxicological and physiological view, it is much
21 more realistic to relate effects to internal (absorbed) O₃ dose rather than to exposure near the leaf
22 or canopy (Fowler and Cape, 1982; Fuhrer et al., 1992; Grünhage et al., 1993, 1999; Legge et al.,
23 1995; Massman et al., 2000; Musselman and Massman, 1999; Pleijel et al., 1995; Runeckles,
24 1974; Taylor et al., 1982; Tingey and Taylor, 1982) (see also Section AX9.4). Theoretically,
25 flux estimates should improve the assessment of O₃ effects, but despite recent attention to this
26 topic, particularly in Europe, it remains difficult to estimate flux in the field outside of
27 experimental sites where continuous measurements of wind speed and other environmental
28 conditions are made. This topic is discussed further below in Section AX9.5.4.5.

29 No simple exposure index can accurately represent all of the numerous factors operating at
different timescales that affect O₃ flux into plants and subsequent plant response (Section

1 AX9.4). Indices of peaks, such as the 2HDM, are not well suited for discerning exposure-
2 response relationships, because they do not capture the effects of lower O₃ concentrations nor the
3 cumulative effects of O₃ on vegetation (Heck and Cowling, 1997; U.S. Environmental Protection
4 Agency, 1996). Therefore, peak indices have not been used in recent decades to develop
5 exposure-response relationships for vegetation. Fortunately, other simple indices have shown
6 substantial correlation with responses such as crop yield under experimental conditions. During
7 the 1980s, the most commonly used indices for expressing O₃ exposure were 7-, 8-, or 12-h
8 daytime average values over the duration of O₃ exposure, which was often 3 months or
9 somewhat less for experimental studies with crops. These indices perform reasonably well for
10 interpreting experimental data on the response of vegetation to O₃, particularly for individual
11 experiments, although they do not explain all of the variation among experiments in
12 retrospective analysis of multiple experiments (Lesser et al., 1990).

13 Since the 1980s, cumulative indices such as the SUM06, AOT40, or W126 that
14 preferentially weight higher concentrations have been used in conjunction with mean indices for
15 developing exposure-response relationships (Tables AX9-14 and AX9-15, and Figure AX9-18).
16 Such indices are often more suitable than mean values, because they are cumulative and because
17 they preferentially weight higher concentrations. Thus, these indices generally provide
18 somewhat better fits to experimental data than do mean indices, especially in retrospective
19 analyses of multiple experiments on multiple species (Lee et al., 1994a; Lee et al., 1994b;
20 Lee and Hogsett, 1999; Tingey et al., 1991). Unfortunately, no single index has been used
21 consistently even in the recent literature, making it difficult to compare results among and
22 between experiments and with ambient exposure data. However, Tables AX9-13 and AX9-21
23 provide summaries of ambient exposure data for several indices that can be compared to the
24 experimental results reviewed in the remainder of this section. Of the cumulative indices that
25 preferentially weight higher concentrations, the SUM06 index has been used most commonly in
26 the U.S. literature, and it was selected in a meeting of scientific experts on O₃ effects on
27 vegetation as suitable for a secondary standard to protect vegetation (Heck and Cowling, 1997).
28 However, it should be noted that the W126 index has been selected for use in protecting
29 vegetation in Class 1 areas (Federal Land Manager's Air Quality Related Values Workgroup
30 (FLAG), 2000). Even in recent studies, O₃ data are often presented using only a seasonal
31 mean index value, and so mean values are frequently presented in this section. Such reporting of

Table AX9-16. Summary of Selected Studies of Ozone Effects on Annual Species

Species	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (Decrease from lowest, %)	Reference
Bean, cv. Pros	OTC	The Netherlands	CF to CF75: 9-h mean = 3-70, AOT40 = 0 to 17.7 ppm•h	62 days	Green pod yield	29 at 9-h mean = 44 (AOT40 = 3.6 ppm•h)	Tonneijck and Van Dijk (1998)
Bean, cv. Lit	OTC	Germany	CF, NF, CF-1×, CF-2×: mean = 1, 14, 15 , 32	3 months	Pod yield	56 (CF, 2×)	Brunschon-Harti et al. (1995)
Bean, cv. Bush Blue Lake 290	OTC	Corvallis, OR	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)	63-65 days	Pod dry weight	51, 57 (early and late season experiments)	Tingey et al. (2002)
Bean, cvs. Tenderette, S156	OTC	Raleigh, NC	CF, 1.4×: 12-h mean = 23, 72	1 years	Pod dry weight	n.s. for Tenderette, 90 for S156	Heagle et al. (1999)
Bean, cvs. R123, Oregon-91, S156	OTC	Raleigh, NC	CF, NF, AA: 12-h mean = 31, 51, 49 in year 2000; 25, 46, 47 in year 2001	2 years	Pod dry weight	n.s. for R123 n.s. for Oregon-91 in 2001, 27 in 2001; 21, 45 for S156	Heagle et al. (1999)
Corn	OTC	Beltsville, MD	CF, +40: 7-h mean = 20, 70	1 years	Grain yield	13	Mulchi et al. (1995) Rudorff et al. (1996c)
Cotton, cv. Deltapine	OTC	Raleigh, NC	CF, 1.5×: 12-h mean = 21, 71	1 years	Seed-cotton weight	22	Heagle et al. (1999)
Cotton, cv. Deltapine	OTC	Raleigh, NC	CF, NF, 1.5×, 12-h mean = 24, 51 , 78	1 years	Seed-cotton weight	21, 49 (NF, 1.5×)	Heagle et al. (1999)
Oat, cv. Vital	OTC	Ostad, Sweden	CF, NF: 7-h mean = 12, 27	1 years	Grain yield	+2 (n.s.)	Pleijel et al. (1994a)

Table AX9-16 (cont'd). Summary of Selected Studies of Ozone Effects on Annual Species

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

Table AX9-16 (cont'd). Summary of Selected Studies of Ozone Effects on Annual Species

Species	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (Decrease from lowest, %)	Reference
Soybean, 3 cvs.	OTC	Raleigh, NC	CF, NF, 1.5×: 12-h mean = 24, 49, 83	4 months	Seed yield	At ambient = 17, 13, 18 (3 cvs.)	Miller et al. (1994)
Soybean, cv. Essex	OTC	Raleigh, NC	CF, NF, 1.5×: 12-h mean = 20, 50, 79	4 months	Seed yield	11, 22 (amb., 1.5×)	Miller et al. (1998)
Soybean, cvs, Essex, Forrest	OTC	Beltsville, MD	CF, NF+: 7-h mean = 24, 58	134 days	Seed yield	Essex = +11 (n.s.), Forrest = 21	Robinson and Britz (2000)
Soybean, cv. Essex	OTC	Raleigh, NC	CF, 1.5×: 12-h mean = 24, 75 (1999); 22, 67 (2000)	164 d (1999), 149 d (2000)	Seed yield	24 (1999), 39 (pots in 2000), 41 (ground in 2000)	Booker et al. (2005)
Timothy	OTC	Sweden	AOT40 = 10, 20, 340; 12-h mean = 20, 152	1 year	Biomass	58	Danielsson et al. (1999)
Watermelon	OTC	Spain	CF (O ₃ = 0), NF in 1988 AOT40 = 5.96 ppm•h, SUM06 = 0.29 ppm•h, in 1989 AOT40 = 18.92 ppm•h, SUM06 = 4.95 ppm•h	2 expts of 1 year	Fruit yield	19, 39 (2 expts)	Gimeno et al. (1999)
Wheat¹, cv. Minaret	OTC	8 sites in N Europe	12-h mean (SD) low = 26.3 (12.2), 12-h mean (SD)-high = 51.4 (18.3) AOT40 mean (SD) low = 6.18 (8.54) ppm•h, AOT40 mean (SD) high = 28.23 (23.05) ppm•h	13 studies of 1 year each	Grain yield	13 (n.s.)	Bender et al. (1999) Hertstein et al. (1999)
Wheat¹	OTC	Sweden	AOT40 0 to 15 ppm•h	7 years	Grain yield	23 at AOT40 = 15 ppm•h	Danielsson et al. (2003)
Wheat, cv. Promessa	OTC	SE Ireland	CF, +50: 12-h total = 5.6, 32.6 ppm•h	3 h/day, 5 d/week, 7 weeks	Grain yield	53	Finnan et al. (1996)
Wheat, cv. Promessa	OTC	SE Ireland	CF, +25: 12-h total = 6.2, 33.4 ppm•h	6 h/day, 5 d/week, 7 weeks	Grain yield	+17	Finnan et al. (1996)

Table AX9-16 (cont'd). Summary of Selected Studies of Ozone Effects on Annual Species

Species	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (Decrease from lowest, %)	Reference
Wheat, cv. Promessa	OTC	SE Ireland	CF, +25, +50: 12-h total = 6.7, 34, 34 ppm•h	+25 = 6 h/day, 5 d/week, +50 = 3 h/day, 5 d/week, both 7 weeks	Grain yield	Amb + 25 = 3 (n.s.); Amb + 50 = 17	Finnan et al. (1996)
Wheat, cvs. Massey, Saluda	OTC	Beltsville, MD	CF, +40: 7-h mean = 19, 20 and 61, 65 (2 years)	2 years	Grain yield	20	Mulchi et al. (1995) Rudorff et al. (1996b)
Wheat, cv. Turbo	OTC	Germany	8-h mean = 5.9, 61.2, 92.5	1 year	Grain yield	14, 40 (mid, high O ₃)	Bender et al. (1994)
Wheat, cv. Turbo	OTC	Germany	8-h mean = 4.7, 86.4	1 year	Grain yield	20	Bender et al. (1994)
Wheat, cv. Turbo	OTC	Germany	7-h mean = 5, 41, 73	1 year	Grain yield	35	Fangmeier et al. (1994)
Wheat, winter, 8 cvs.	OTC	Raleigh, NC	12-h mean = 27, 47, 90	2 months	Grain yield	5 (n.s.)	Heagle et al. (2000)
Wheat, winter, 8 cvs	OTC	Raleigh, NC	12-h mean = 22, 38, 74	2 months	Grain yield	16 (n.s.)	Heagle et al. (2000)
Wheat, cv. Drabant	OTC	Finland	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	2 years	Grain yield	At highest O ₃ = 13 each year	Ojanpera et al. (1998)
Wheat, cv. Riband	Open Air	Northumberland, UK	AOT40 for Mar to Aug 93 = 3.5, 6.2 ppm•h	1 year, overwinter	Grain yield	13	Ollerenshaw and Lyons (1999)

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 AX9-17. Summary of Selected Studies of the Effects of Ozone on Perennial Herbaceous Plants

Species	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (Decrease from lowest, %)	Reference
Alfalfa, cvs. Apica, Team	OTC	Quebec, Canada	12-h mean: 1991 = 6, 39, 49, 110; 1992 = 0, 34, 42, 94	3 months in each of 2 years	Biomass	For NF: Apica = 31, 21; Team = 14, 2 (n.s.)	Renaud et al. (1997)
Bahia grass	OTC	Auburn, AL	12-h mean = 22, 45, 91	24 weeks	Biomass at ambient O ₃ for 1st, 2nd cutting of early and late season plantings.	34, 29 (n.s.), +6 (n.s.), 9 (n.s.)	Muntifering et al. (2000)
Bent grass (<i>Capillaris</i> sp.)	OTC	United Kingdom	AOT40 = 0.8-15.0 ppm•h	8 h/day for 3 months	Biomass, in competition with 3 other spp. Total biomass in uncut pots, aboveground biomass in cut pots (cut every 14 days).	8 (uncut), +18 (cut)	Ashmore and Ainsworth (1995)
Blackberry	Large OTC	Alabama	1994: AOT40 = 2-112 ppm•h, SUM06 = 1-162 ppm•h, 1995: AOT40 = 3-83 ppm•h, SUM06 = 0-132 ppm•h	7 months in 1994, 6 months in 1995	Percent canopy cover (grown in old field community), biomass ripe fruit number.	+124 for cover, n.s. for biomass, 28% for ripe fruit number but sig. chamber effect.	Barbo et al. (1998) Chappelka (2002)
Clover, white	Ambient air	MA, OR, NC, CA (2 sites) and VA	SUM06 for 6-h/day = 10.2-39.4 ppm•h, AOT40 for 12-h/day = 0.6-50.1 ppm•h	2 growing seasons	Biomass ratio (sensitive/resistant)	4 at 6-h SUMO6 = 39.4 ppm•h; 12 h AOT40 = 50.1 ppm•h	Heagle and Stefanski (2000)
Clover, white	Ambient air	14 European sites	AOT40 for 28 d = 0-12 ppm•h	3 growing seasons	Biomass ratio sensitive/resistant)	5 at AOT40 for 28 days = 0.9-1.7 ppm•h	Mills et al. (2000)
Clover, white	OTC	United Kingdom	AOT40 = 0.8-15.0 ppm•h	8 h/day for 3 months	Biomass, in competition 3 other spp. Total biomass in uncut pots, aboveground biomass in cut pots (cut every 14 days).	18 (uncut), 40 (cut)	Ashmore and Ainsworth (1995)
Clover, white and red	OTC	Switzerland	CF, NF, NF+, NF++: 12-h mean = 21, 39 , 47, 65	3.5 months/ year for 2 years	Biomass, in managed pasture	24, 26, 52	Fuhrer et al. (1994)
Clover, white, cv. Menna	OTC	Italy	CF, NF: AOT40 = 0.1; 8.9 ppm•h, 7-h mean = 24, 53	2 months	Biomass	20	Fumagalli et al. (1997)

Table AX9-17 (cont'd). Summary of Selected Studies of the Effects of Ozone on Perennial Herbaceous Plants

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

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 AX9-18. Summary of Selected Studies of Ozone Effects on Deciduous Trees and Shrubs

Species	Age	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (Decrease from lowest, %)	Reference
Ash, European		OTC	Hampshire, UK	NF, NF+: Mean = 17.7, 44.1; AOT40 for 24h = 1.9, 59.9 ppm•h	3 years for day 100 to day 162	Growth and biomass of organs	n.s.	Broadmeadow and Jackson (2000)
Ash, European	Seedling	OTC	Switzerland	0.5×, 0.85×, 1×, 0.5×+30: AOT40 = 0.1, 3.4, 7.1, 19.7 ppm•h	5 months	Biomass	26 at 1×, 50 at 0.5× + 30	Landolt et al. (2000)
Aspen	Cutting, Seedling	OTC	Michigan	CF, 1×, 2×: 3 months 7-h mean for 1990 = 7, 43, 63 ; for 1991 (square wave exposure) = 11, 45, 66	98 days	Total biomass of 3 clones and seedlings	For 1990: 2-22 at 1x for clones (mean = 16), 14 for seedlings. For 1991: 23-39 at 1x for clones (mean = 30)	Karnosky et al. (1996)
Aspen	Cutting	OTC	Michigan	CF, 1×, 2×: SUM00 = 11, 58, 71 ppm•h	98 days	Total biomass	25-38 at 1×	Dickson et al. (2001)
Aspen	Cutting	FACE	Wisconsin	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	7 years (only 4 years of O ₃ data reported)	Volume (d2*h)	21 after 3 years, 14 after 7 years at 1.5x	Isebrands et al. (2001)
Aspen	Cutting	Large OTC	New York	1×, 1.7×, 3×: SUM06 = 1, 20, 62 ppm•h; 9-h mean = 40, 74, 124	92 days	Shoot biomass	14, 25 for 2 clones at 1.7×	Yun and Laurence (1999)
Aspen	First year	OTC	Pennsylvania	8-h mean = 39, 73	11 weeks	Biomass	14-30 for 3 of 6 N treatments	Pell et al. (1995)
Beech, European		OTC	Switzerland	0.5×, 0.85×, 1×, 0.5×+30: AOT40 = 0.1, 3.4, 7.1, 19.7 ppm•h	5 months	Biomass	6 at 1×, 30 at 0.5×+30	Landolt et al. (2000)
Beech, European	Seedling	OTC	Belgium	CF, NF, +30: 8-h mean = 5. 29, 33; AOT40 = 0. 4.06, 8.88 ppm•h	23 April - 30 Sept	Growth	No effect	Bortier et al. (2000a)

Table AX9-18 (cont'd). Summary of Selected Studies of Ozone Effects on Deciduous Trees and Shrubs

Species	Age	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (Decrease from lowest, %)	Reference
Beech, European	Seedling	Growth chamber	Belgium	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}	7 episodes of 5 days	Biomass, diameter	No effect	Bortier et al. (2001)
Beech, European	0-3 years	OTC	Switzerland	AOT40 for 24 h/days = 4-73 ppm•h	1-3 years	Total biomass	20 at AOT40 for 24 h = 32 ppm•h	Braun and Fluckiger (1995)
Beech, Japanese	4 years	Growth chamber	Japan	CF, +60 ppb for 7 h/day	156 days	Total biomass	19	Yonekura et al. (2001)
Birch, silver	Sapling	FACE	Finland	AOT40 = 1, 15 ppm•h; 7-h mean = 26, 40	5 years	Biomass	34 for root, n.s. for stem	Oksanen et al. (2001)
Birch, silver	Sapling	OTC	Sweden	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	2 years	Total biomass	Total biomass n.s. at NF+, 22 at NF++; root biomass 30 at NF++	Karlsson et al. (2003)
Birch, [<i>B. pubescens</i>]	Seedling	Chamber in glasshouse	Norway	AOT40 = 0.1, 2.5, 7.1, 7.4, 17.8, 19.8 ppm•h	40 days	Biomass	Sig. decrease in root at AOT40 = 2.5 ppm•h, shoot at 7.1 ppm•h	Mortensen (1998)
Birch, paper	Sapling	FACE	Wisconsin	Ambient, 1.5x: 4 y Ambient 12-h mean = 34.6, 36.9, 36.0, 36.6; 4y 1.5x 12-h mean = 54.5, 51.1, 48.9, 52.8	7 years (only 4 y of O ₃ data reported)	Volume (d2•h)	No effect	Isebrands et al. (2001)
Cherry, black	2 years	OTC	Norris, TN	CF, 1x, 2x: 7-h mean = 21, 50, 97	April to August	Biomass	No effect	Samuelson, 1994)

Table AX9-18 (cont'd). Summary of Selected Studies of Ozone Effects on Deciduous Trees and Shrubs

Species	Age	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (Decrease from lowest, %)	Reference
Cherry, black	Seedling	OTC	GSMNP ¹	CF, 1×, 1.5×, 2×: SUM06 = 0-40.6 ppm•h, AOT40 = 0.03-28.3 ppm•h	76 days	Biomass	n.s. at 1x and 1.5×, 38 at 2×	Neufeld et al. (1995)
Cherry, black	Seedling	OTC	GSMNP ¹	CF, 0.5×, 1×, 1.5×, 2×: SUM06 = 0-53.7 ppm•h; AOT40 = 0-40.4 ppm•h	140 days	Biomass	n.s. at 1× and 1.5×, 59 at 2×	Neufeld et al. (1995)
Cherry, black	1 year	OTC	Delaware, OH	CF, 0.5, 1, 1.5, 2×: SUM00 in 1990 = 17-107 ppm•h, in 1991 = 31-197 ppm•h	2 years (in 1990 for 3.5 months, 1991 for 4 months)	Total biomass	no effect at 1× and 1.5×, 32 at 2×	Rebbeck (1996)
Cherry, black	Seedling	OTC	Pennsylvania	CF, 0.75×, 0.97×: 7-h mean = 39 to 46 , SUM06 = 0- 10.34 ppm•h	3 years for 17 weeks	Total biomass	6 at 0.75×, 14 at 0.97×	Kouterick et al. (2000)
Cottonwood, Eastern	Cutting	Ambient, in buried pots with irrigation	In and within 100 km of New York City, NY	12 h mean = 23-49 ppb	2 months each year, 3 10-years experiments	Total biomass	33% decrease at 38 ppb compared to 23 ppb	Gregg et al. (2003)
Grape	3 years	OTC	Austria	CF, 1×, +30, +50: (AOT40 = 0-50 ppm•h	2 years (preflowering, past harvest)	Fruit yield	Calculated 10 at AOT40 = 27 ppm•h	Soja et al. (1997)
Oak	Seedling	OTC	Hampshire, UK	NF, NF+: Mean = 17.7 , 44, AOT40 for 24h = 1.9 , 59.9 ppm•h	3 years for day 100 to day 162	Biomass of organs	30 for total biomass	Broadmeadow and Jackson (2000)
Oak, red	Seedling	OTC	Norris, TN	SUM06 for 3 years = 0, 29, 326 ppm•h; SUM00 for 3 years = 147, 255 and 507 ppm•h	3 years	Total biomass	n.s.	Samuelson et al. (1996)

Table AX9-18 (cont'd). Summary of Selected Studies of Ozone Effects on Deciduous Trees and Shrubs

Species	Age	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (Decrease from lowest, %)	Reference
Oak, red	30 years	OTC	Norris, TN	SUM06 for 3 years = 0, 29, 326 ppm•h; SUM00 for 3 years = 147, 255 and 507 ppm•h	3 years	Stem increment	n.s. despite 50% reduction in net photosynthesis	Samuelson et al. (1996)
Maple, red	2 years	OTC	Norris, TN	CF, 1×, 2× : 7-h mean = 21, 50, 97 ppm•h	April to August	Biomass	No effect	Samuelson (1994)
Maple, sugar	1 year	OTC	Delaware, OH	CF, 0.5, 1.5, 2×: UM00 in 1990 = 17 to 107 ppm•h, in 1991 = 31 to 197 ppm•h	2 years (in 1990 for 3.5 months, 1991 for 4 months)	Total biomass	n.s., but linear trend	Rebbeck (1996)
Maple, sugar	Seedling	Large OTC	Ithaca, NY	CF, 1×, 1.5×, 2×: 3 years SUM00 = 148 to 591 ppm•h; daytime mean = 19.7 to 40.7	3 years for 134, 128, 109 days	Biomass	No effect	Laurence et al. (1996)
Maple, sugar	Seedling	Large OTC	Ithaca, NY	1×, 1.7×, 3×: 3 years 12-h mean = 38, 69, 117	3 years for 109, 143, 116 days	Total biomass	For 1.7× and 3×: 21, 64 in low light, 26 and 41 in high light	Topa et al. (2001)
Maple, sugar	Sapling	FACE	Wisconsin	Ambient, 1.5×: 4 y Ambient 12-h mean = 34.6, 36.9, 36.0, 36.6; 4y 1.5x 12-h mean = 54.5, 51.1, 48.9, 52.8	7 years (only 4 y of O ₃ data reported)	Volume (d2•h)	18	Isebrands et al. (2001)
Plum, Casselman	Sapling	Large OTC	Fresno, CA	CF, 1×, +O ₃ : 12-h mean = 31, 48, 91	4 years	Stem increment, fruit yield	Fruit yield 16 at 1×, stem +14 at +O ₃	Retzlaff et al. (1997)
Poplar, black	Seedling	OTC	Belgium	CF, NF, +30: 8-h mean = 5, 29, 33; AOT40 = 0, 4, 8.9 ppm•h	23 April - 30 Sept	Diameter, height	29 for diameter in NF+, no effect on height	Bortier et al. (2000b)

Table AX9-18 (cont'd). Summary of Selected Studies of Ozone Effects on Deciduous Trees and Shrubs

Species	Age	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (Decrease from lowest, %)	Reference
Poplar, hybrid (<i>P. tremuloides</i> × <i>P. tremula</i>)	0 year	FACE	Finland	AOT40 = 0.07, 1.6 ppm•h; 7-h mean = 30, 38	2 months	Biomass, height	n.s. for biomass, 6 for height	Oksanen et al. (2001)
Poplar, hybrid	Cutting	OTC	Michigan	CF, CF+100: 12, 48 ppm•h	60 days	Total biomass	46 for average of 5 clones	Dickson et al. (1998)
Poplar, hybrid	Cutting	OTC	Michigan	CF, CF+100: 12, 48 ppm•h	60 days	Total biomass	46 for average of 5 clones	Dickson et al. (1998)
Yellow-poplar	1 year	OTC	Delaware, OH	CF, 0.5×, 1.5×, 2×: SUM00 in 1990 = 17 to 107 ppm•h, in 1991 = 31 to 197 ppm•h	2 years (in 1990 for 3.5 months, 1991 for 4 months)	Total biomass	No effect	Rebbeck (1996)
Yellow-poplar	1- 7 year	Large OTC	Delaware, OH	CF, 0.5×, 1.5×; SUM00= 145, 583, 861 ppm•h ; SUM06 = 0.3, 228.7, 661.8 ppm•h over 5 years	5 years	Total biomass	No effect	Rebbeck (1996)

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

Species	Age	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (decrease from lowest, %)	Reference
Fir, Douglas	Seedling	Open air	British Columbia	12 trts: 12-h mean 1988 = 18-41; 1989 = 27-66	1988 = 92 days; 1989 = 101 days	Second flush biomass	Calculated 55 at highest exposure	Runeckles and Wright (1996)
Hemlock, eastern	Seedling	OTC	GSMNP ¹ , TN	CF to 2×: SUM06 = 0.2-108.1 ppm•h, AOT40 = 0.2-63.9 ppm•h	3 years	Biomass	No effect	Neufeld et al. (2000)
Pine, loblolly	12 weeks	OTC	Oak Ridge, TN	CF to 2×: 24-h summer = 74, 137, 169, 206, 284 ppm•h	3 months	Biomass	14 in 1× (avg for all families)	McLaughlin et al. (1994)
Pine, loblolly	1 year	OTC	Alabama	1994: AOT40 = 2-112 ppm•h, SUM06 = 10-162 ppm•h, 1995: AOT40 = 3-83 ppm•h, SUM06 = 0-132 ppm•h	2 years, April to October	Dry weight, height, diameter	n.s.	Barbo et al. (2002)
Pine, loblolly	3 years	OTC	Raleigh, NC	Ambient, CF, NF, 1.5×., 2.5×: 12-h mean = 54 , 29, 47, 76, 98	5 months	Height, diameter, needle length	No effect on stem height or diameter, decrease in needle length	Anttonen et al. (1996)
Pine, loblolly	4 weeks	Large OTC	Auburn, AL	CF, 1×, 2×: 12-h mean = 13, 47,, 98 ppm•h in 1998; 12, 44, 97	2 12-week experiments	Shoot biomass, root biomass, foliar injury	Shoot = 15 in 1x, 22 in 2x in both years; Root = 26 in 2x in both years; Foliar sig. greater in 1x in 1999 and 2x in both years.	McLaughlin et al. (1994)
Pine, ponderosa	Seedling	OTC	Corvallis, OR	For CF 12-h SUM06 = 0 ppm•h; for +03 12-h SUMO6 = 22, 27, 31 ppm•h for 3 years	3 years: 16 weeks, 16 weeks, 14 weeks	Total biomass	No effect without grass, 25 with grass present	Andersen et al. (2001)

Table AX9-19 (cont'd). Summary of Selected Studies of Ozone Effects on Evergreen Trees and Shrubs

Species	Age	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (decrease from lowest, %)	Reference
Pine, ponderosa	39 to 45 years	Ambient gradient	CA	24-h mean for 3 weeks late July and early August for 1993 and 1994 = 70-90 ppb	Ambient gradient	Fine and medium root growth	85 at most polluted site.	Grulke et al. (1998)
Pine, ponderosa	Seedling	OTC	CA	CF, 1×, 2×: 24-h mean approx. 20, 60 , 120		Total biomass	n.s.	Takemoto et al. (1997)
Pine, Scots		OTC	Hampshire, UK	NF, NF+: Mean = 17.7 , 44.1; 24-h AOT40 = 1.9 , 59.9 ppm•h	3 years for 62 days	Total biomass	15	Broadmeadow and Jackson (2000)
Pine, Scots	3-6 years	Free air	Finland	Amb, +O ₃ : AOT40 = 0-1 , 2-13 ppm•h	3 years	Biomass	No effect	Kainulainen et al. (2000)
Pine, Scots	Seedling	OTC	Switzerland	0.5×, 0.85×, 1×, 0.5×+30: AOT40 = 0.1, 3.4, 7.1 , 19.7 ppm•h	5 months	Biomass	14 at 1×, 22 at 0.5×+30	Landolt et al. (2000)
Pine, Scots	3 years	OTC	Finland	CF, 1×, +O ₃ : 24 h AOT40 for 2 years = 0.5, 6 , 73 ppm•h	2 years (4 months each)	Biomass	No effect	Utriainen et al. (2000)
Pine, Scots	3 years	Free air	Finland	1×, +O ₃ : 24 h AOT40 for 2 years = 2 , 37 ppm•h	3 years (3-4 months each)	Root and shoot biomass	32 only for root biomass in high N treatment	Utriainen and Holopainen (2001)
Pine, Table Mountain	Seedling	OTC	GSMNP ² , TN	CF to 2×: SUM06 = 0.2-116.4 ppm•h, AOT40 = 0.2-71.7 ppm•h	3 years	Biomass	Slight decrease in older needle mass only	Neufeld et al. (2000)
Pine, Virginia	Seedling	OTC	GSMNP ² , TN	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	1-2 years (3 expts)	Biomass	No effect	Neufeld et al. (2000)

Table AX9-19 (cont'd). Summary of Selected Studies of Ozone Effects on Evergreen Trees and Shrubs

Species	Age	Facility	Location	O ₃ Concentration (units are ppb unless otherwise specified) ¹	Duration	Variable	Response (decrease from lowest, %)	Reference
Sequoia, giant	125 years	Branch chamber	California	0.25×, 1×, 2×, 3×: 24-h SUM00 approx. 10, 85, 180, 560 ppm•h	61 days	Branch growth	No effect	Grulke et al. (1996)
Spruce, Norway	4-7 years	Open air	Finland	Amb, +O ₃ : AOT40 = 0 1 , 2-13 ppm•h	3 years	Biomass	No effect	Kainulainen et al. (2000)
Spruce, Norway	3-7 years	OTC	Sweden	CF, 1.5×: 12-h mean for 4 years = 12, 44; AOT40 = 2, 23 ppm•h	4 years	Total biomass	8	Karlsson et al. (2002)
Spruce, Norway	Seedling	OTC	Switzerland	0.5×, 0.85×, 1×, 0.5×+30: AOT40 = 0.1, 3.4, 7.1, 19.7 ppm•h	5 months	Biomass	n.s.	Landolt et al. (2000)
Spruce, Norway	0-3 years	OTC	Switzerl and	AOT40 for 24 h for 1 to 3 years = 22 to 63 ppm•h	1-3 years	Total biomass	n.s.	Braun and Fluckiger (1995)
Spruce, Norway	3 to 7 years	OTC	Sweden	CF, 1×, 1.5×: AOT40 daylight for 4 years = 1, 16 , 79 ppm•h	4 years	Biomass	5.3 at 1.5x	Wallin et al. (2002)
Spruce, red	Saplin g	Large OTC	Ithaca, NY	CF, 1×, 1.5×, 2×: total for 4 years = 211 to 569 ppm•h; daytime mean = 21-71	4 years: 98-124 days/year	Biomass	No effect	Laurence et al. (1997)

¹ Values for ambient or NF treatments are indicated in bold.² Great Smoky Mountains National Park.

Table AX9-20. Ethylene Diurea Effects on Vegetation Responses to Ozone

Species	Description	EDU Application	Ozone Exposure	Effects of EDU	Reference
Bean, cv. Lit	10-cm pots in OTCs in Germany	Soil drench 200 mL of 150 ppm solution per plant every 14 days	CF, NF, CF-1×, CF-2×: mean = 1, 14, 15, 32 ppb	O ₃ reduced pod, shoot, and root mass. EDU increased root, leaf, and shoot mass, but a significant interaction with O ₃ occurred only for root weight.	Brunschon-Harti et al. (1995)
Bean, cv. BBL-290	2 expts in 5.5 L pots in OTCs with 4 O ₃ treatments	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	2 expts with CF, NF and 2 constant additions of O ₃ . 7-h mean O ₃ (ppb) for Expt 1 = 34, 70, 95, 121; Expt 2 = 19, 42, 74, 106	Visible injury and reduced total biomass or yield, even in CF treatment. Within an O ₃ treatment, sometimes increased yield (Expt 2 only).	Miller et al. (1994)
Bean, cv. Bush Blue Lake 290, Bush Blue Lake 274, lines S156, R123	Field-grown in fine sandy loam in Massachusetts, in 2 one-year experiments.	Foliar spray at 300 ppm every 7 days between full expansion of primary leaves and pod senescence.	Ambient, with 181 and 141 h > 40 ppb, 74 and 95 h > 60 ppb, and 23 h > 80 ppb in 2001 and 2002 respectively	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.	Elagöz and Manning al. (2005a)
Bean	Pots with potting mix at 3 locations in Spain (2 years at 1 site)	Soil drench of 200 mL of increasing concentrations of 100, 150, 200, 250 ppm every 14 days (4-10 mg l:1 soil)	AOT40 = 0.4-1.8 ppm-h	0 to 50% increase in pod mass, but did not restore yield at sites with higher O ₃ .	Ribas and Penuelas (2000)
Bean, cv. Lit	Pots with potting mix at 4 sites in the Netherlands	Soil drench of 200 mL of increasing concentrations of 100, 150, 200, 250 ppm every 14 days (4-10 mg l:1 soil)	AOT40 = 0.64-0.98, 7-h mean = 49-55 ppb	Average 20% yield increase at all sites.	Tonneijck and Van Dijk (1997)
Bean, cv. Lit	Pots with potting mix at 1 site in Belgium	Soil drench of 200 mL of increasing concentrations of 100, 150, 200, 250 ppm every 14 days (4-10 mg l:1 soil)	AOT40 = 0.81 ppm-h	16% yield increase.	Vandermeiren et al. (1995)

Table AX9-20 (cont'd). Ethylene Diurea Effects on Vegetation Responses to Ozone

Species	Description	EDU Application	Ozone Exposure	Effects of EDU	Reference
Clover, subterranean	Plants in 10-cm pots at 4 rural sites in the Netherlands for 3 years	100 ml of 150 ppm solution as soil drench every 14 days for 2 months	AOT40 = 0-0.56 ppm-h for 4-week periods	Injury, but not leaf biomass was affected by EDU and O ₃ exposure.	Tonneijck and Van Dijk (2002)
Clover, white	15-cm pots in field, well watered, 12 locations throughout Europe, 3 years	100 mL of 150 ppm solution as soil drench every 14 days for 3 months	AOT40 (28 days) = 0-20 ppm-h	Change in biomass ratio, weak linear relationship ($r^2 = 0.16$) stronger relationship using ANN and climatic factors	Ball et al. (1998)
Clover, white, cv. Menna	2 expts, 10-cm pots in field in Italy, see also companion OTC expt	100 mL of 150 ppm solution as soil drench every 14 days for 2 months	AOT40 = 15.5, 12.1 ppm•h; 7-h mean = 69, 60	n.s.	Fumagalli et al. (1997)
Clover, white, cv. Menna	2 expts, 10-cm pots in OTCs in Denmark	Soil drench 100 mL of 150 ppm solution every 14 days	CF, NF, NF+ 25, NF+50 ppb, O ₃ exposure not reported	No effect of EDU despite highly significant effect of O ₃ on above-ground biomass	Mortensen and Bastrup-Birk (1996)
Poplar, hybrid	Stem injections, field, cuttings, 1 or 2 years	Approx. 125 or 250 mg/leaf (low, high EDU treatments) 5 times every 14 days	1991: 7-h mean = 56, AOT40 = 23; 1992: 7-h mean = 59, AOT40 = 27	No effect on biomass; 6%, 12% more severely O ₃ damaged leaves in high EDU for 2 years	Ainsworth et al. (1996)
Pine, loblolly	1 year old half-sib seedlings in field in TX for 3 years	150, 300, 450 ppm every 14 days	1995, 1996, 1997, no. h > 40 ppb = 1723, 2297, 2052; no. h > 60 ppb = 378, 584, 528; peak = 113, 102, 118	For EDU 450 trt, above-ground biomass increased approx 46% (n.s. in other treatments)	Manning et al. (2003)
Radish, cv. Cherry Belle	Plants in pots in potting mix exposed for 5 weeks in southern Sweden.	Soil drench containing 20 mg EDU applied 2 times, 14 days apart	24-h mean = 31 ppb, 7-h mean = 36 ppb, AOT40 = 1.3 ppm-h.	24% increase in hypocotyl mass, 18% increase in shoot mass	Pleijel et al. (1999b)

1 mean indices should not be interpreted as a preference for them, but rather as a limitation in the
2 data reported in the literature. Additional information about O₃ exposure for individual
3 experiments, including the number and type of O₃ treatments (addition of a constant
4 concentration of O₃ or an amount proportional to ambient levels), and duration, are reported in
5 Tables AX9-16 through AX9-19.

6 Since the 1996 O₃ AQCD, the use of the AOT40 index has become quite common in
7 Europe for identifying and mapping areas of exceedance, but it has not been used much in the
8 United States. Thus, studies reporting O₃ exposure only as AOT40 values are presented in tables
9 summarizing effects on annual, herbaceous perennial, and woody vegetation. However, such
10 studies are not as commonly cited in the text of this section, because AOT40 summary data
11 on O₃ exposures in the United States are rarely available. This lack makes it difficult to compare
12 experimentally derived exposure-response data expressed as AOT40 to ambient U.S. O₃
13 exposures. The development of critical levels in Europe has been based primarily on the AOT40
14 index, so this index is discussed in that context.

15 In addition to peak weighting, there is also evidence that the timing of exposure during
16 plant growth is important. For example, the greatest effects on grain yield are due to exposure
17 during grain filling, rather than earlier or later in the growing season (Lee et al., 1988; Pleijel
18 et al., 1998; Soja et al., 2000; U.S. Environmental Protection Agency, 1996; Younglove et al.,
19 1994). A recent study grew bush bean in OTCs with CF or above-ambient O₃ using exposure
20 dynamics typical of the Midwestern United States for three time periods: (1) the entire season,
21 (2) the period prior to anthesis, (3) during pod filling and maturation (Tingey et al., 2002).
22 Ozone exposure prior to anthesis reduced growth by less than 1% per ppm-h (SUM06) while
23 exposure during pod filling and maturation reduced growth by 4 to 7%. A meta-analysis of
24 53 studies of O₃ effects on soybean found that O₃ had greater effects with increases in
25 developmental stage, with the greatest effect during seed filling (Morgan et al., 2003). The
26 importance of respite times was discussed in the previous criteria documents (U.S.
27 Environmental Protection Agency, 1978, 1986, 1996) but remains difficult to quantify (Section
28 AX9.4). Even when some of these aspects of O₃ exposure can be elucidated, it is difficult to
29 apply this knowledge to developing exposure-response relationships based on data in the
30 scientific literature, because O₃ exposure is often reported only in the form of a summary index
31 such as a 12- or 24-h mean, SUM06, or AOT40.

1 Table AX9-13 presents summaries of ambient O₃ exposure patterns in the United States for
2 1982 to 1991 for several indices including the 7-h mean and SUM06. More recent summaries
3 for the entire United States for these indices are not available, but Table AX9-21 summarizes
4 more recent data for the central and eastern United States. As shown in Table AX9-21, from
5 1989 to 1995, mean 12-h 3-month SUM06 values (in ppm-h) at 41 rural sites in the Clean Air
6 Status and Trends Network were 31.5 for the Midwest, 18.9 for the Upper Midwest, 33.2 for the
7 Northeast, 13.2 for the Upper Northeast (New Hampshire, Maine), 34.5 for the South-Central,
8 and 19.2 for the Southern Peripheral subregions (Baumgardner and Edgerton, 1998). These
9 results are important because these sites were selected to represent rural areas, while many other
10 monitoring sites represent urban or suburban areas. For these same subregions, W126 values
11 ranged from 12.8 to 25.6 ppm-h. From 1989 to 1995, O₃ concentrations decreased about 5% for
12 daily and 7% for weekly values for most of these sites, after adjusting for meteorological
13 conditions (Holland et al., 1999). These trends were statistically significant at about 50% of the
14 sites ($p \leq 0.05$). However, because the trend analysis was intended to examine the efficacy of O₃
15 emissions controls, the trends were adjusted for meteorological conditions. Thus, they do not
16 reflect the actual trends in O₃ exposure over time.

17 18 **AX9.5.4 Effects of Ozone on Annual and Biennial Species**

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

22 An extensive search of the literature was performed using several electronic databases to
23 identify scientific articles containing quantitative information on both the amount of O₃ exposure
24 and its effects on vegetation. Greater emphasis is placed on studies with longer duration with O₃
25 exposure concentrations and environmental conditions that were as similar as possible to
26 ambient conditions. Many of the studies reviewed herein were conducted in OTCs. In the
27 United States, nearly all of such studies have used the type of OTC developed by Heagle et al.
28 (1973). For the few studies in the U.S. that used other types of OTCs, they are described briefly
29 in the text. In Europe, a wide variety of styles of OTCs have been used. See Section AX9.1 for
30 further information about the use of OTCs. The emphasis in this subsection is on quantifying
31 exposure-response relationships for annual plants, with a focus on the response of above-ground

1 biomass and yield of species grown as crops or occurring as native or naturalized species in the
2 United States. Emphasis is placed on studies not included in the 1996 AQCD (U.S.
3 Environmental Protection Agency, 1996), including a few studies published prior to 1996.
4 However, an attempt is made to compare the results of these more recent studies of individual
5 species to those reviewed in the 1996 AQCD.

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

8 Most research on the effects of O₃ on herbaceous species has evaluated growth, biomass, or
9 yield of commercial portions of crop or forage species. It is well established that reproductive
10 organs such as seeds may be particularly sensitive to injury or biomass reductions due to O₃, as
11 reviewed by (Black et al., 2000). As discussed in Section AX9.3, numerous analyses of
12 experiments conducted in OTCs and with naturally occurring gradients demonstrate that the
13 effects of O₃ exposure vary depending on the growth stage of the plant. Plants grown for seed or
14 grain are often most sensitive to exposure during the seed or grain-filling period (Lee et al.,
15 1988; Pleijel et al., 1998; Soja et al., 2000; Younglove et al., 1994), whereas plants grown for
16 biomass production, such as alfalfa, may be sensitive throughout the growth period (Younglove
17 et al., 1994). However, because different species are sensitive during different periods of their
18 growth and, because planting or germination dates vary throughout large regions even for a
19 single species, no single phenological weighting scheme can appropriately and practically
20 represent all vegetation in all locations in the United States. For natural populations, reductions
21 in seed yield might be particularly important if subsequent seedling establishment is
22 compromised by O₃.

23 Green beans (cv. Pros) were grown in pots in OTCs in the Netherlands for 62 days and
24 exposed to 6 treatments consisting of constant O₃ additions to CF chambers (see Section AX9.1)
25 for 9 h/day (Tonneijck and Van Dijk, 1998). Bean yield response to O₃ was nonlinear, with an
26 apparent threshold near the CF30 (charcoal filtered with a constant addition of 30 ppb O₃)
27 treatment with a 9-h mean O₃ concentration of 28 ppb and an AOT40 value of 0.1 ppm-h). Yield
28 was reduced by 29% at a 9-h mean value of 44 ppb corresponding to an AOT40 value of 3.6
29 ppm-h (Table AX9-16). Beans were grown in pots in OTCs for 3 months with the following O₃
30 treatments: CF, nonfiltered (NF), CF with O₃ added up to ambient, and CF with 2×-ambient O₃
31 (Brunschon-Harti et al., 1995). Ozone reduced pod mass by 56% with a mean concentration of

1 32 ppb in the 2× ambient treatment as compared with 1 ppb in the CF treatment (daily averaging
2 time not reported). A second treatment factor in this experiment was addition of EDU, as
3 discussed below under the heading “Studies Using Ethylene Diurea as a Protectant.” Bush beans
4 (cv Bush Blue Lake 290) were grown in pots in OTCs in Corvallis Oregon for 63 or 65 days in
5 two experiments, one from May to July and one from August to October (Tingey et al., 2002).
6 Plants were exposed to either CF air or CF air with above-ambient O₃ with temporal frequency
7 and exposure dynamics typical of the Midwestern United States, with SUM06 values of 0.0 for
8 the CF treatment and 75.7 or 68.4 ppm·h for the two experiments. Ozone exposure reduced pod
9 dry weight by 51 and 57% in the two experiments. The sensitive cultivar S156 and the more
10 resistant cultivar Tenderette were grown in pots in OTCs in Raleigh, NC at either CF (12-h mean
11 of 23 ppb) or 1.4× (12-h mean of 72 ppb) (Heagle et al., 2002). At final harvest, the dry weight
12 of the sensitive cultivar was reduced 90% by the O₃ treatment, but the more resistant cultivar
13 was not reduced. In two additional OTC experiments during 2000 and 2001 in Raleigh, NC, the
14 sensitive cultivar S156, the moderately sensitive cultivar Oregon-91, and the more resistant
15 cultivar R123 were grown in CF, NF, and ambient air treatments (Fiscus et al., 2005). For the NF
16 treatment of the two experiments, the yield of S156 was reduced 21% and 45%, that of Oregon-
17 91 by 27% only in 2001, while R123 was not significantly reduced. These yield reductions are
18 greater than those previously reported in four similar studies summarized in the 1996 AQCD
19 (Table 5-25 of U.S. Environmental Protection Agency, 1996). Greater sensitivity in the more
20 recent experiments may be due to cultivar differences or other differences in experimental
21 protocols.

22 In a study with OTCs on silty loam soil in Beltsville, MD, corn yield was reduced by 13%
23 with exposure to a 7-h mean concentration of 70 ppb O₃ compared to a CF treatment with a 7-h
24 mean concentration of 20 ppb (Mulchi et al., 1995; Rudorff et al., 1996a,c). In this study,
25 different amounts of O₃ were added above ambient levels for 5 days follows: 20, 30, 40, 50, 60
26 ppb, except that O₃ was not added to exceed a total concentration of 120 ppb (Rudorff et al.,
27 1996a,c).

28 In two studies conducted in Raleigh, NC, cotton (cv. Deltapine 51) was grown in pots and
29 exposed to CF and 1.5× (nonfiltered, see Section AX9.1) O₃ in one year, and CF, NF, and
30 1.5×-ambient O₃ in the second year, with ambient and elevated CO₂ concentrations (Heagle
31 et al., 1999) (Table AX9-16). In the first year, yield decreased by 22% with 1.5×-ambient O₃

1 (12-h mean value of 71 ppb). In the second year, yield decreased by 21% and 49% with
2 exposure to ambient or 1.5× ambient O₃ (12-h mean values of 51 and 78 ppb, Table AX9-16).
3 Increased CO₂ levels prevented or reduced this yield suppression (Heagle et al., 1999). These
4 yield reductions are similar to those reported previously in four similar studies summarized in
5 the 1996 AQCD (Table 5-25 of U.S. Environmental Protection Agency, 1996).

6 In a study of oats in OTCs in southern Sweden, exposure to ambient (NF) O₃ did not affect
7 grain yield (Pleijel et al., 1994a). Ambient O₃ concentration expressed as a 7-h mean was
8 27 ppb, with only 1 h greater than 80 ppb and none above 90 ppb.

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

24 The effect of an intermittent constant addition of O₃ using a free air exposure system in
25 Northumberland, UK was investigated with the oilseed rape cultivar Eurol (Ollerenshaw et al.,
26 1999). Ozone was added for 6 h/day for 17 days. The ambient treatment had a mean value of 30
27 ppb and the O₃ addition treatment had a mean of 77 ppb. After overwintering, O₃ was added for
28 32 days for 7 h/day between May and June (mean values of 31 and 80 ppb). Yield was reduced
29 by 14% despite the lack of any foliar symptoms.

30 Field fumigation chambers ventilated with fans on both ends were used to assess effects of
31 five O₃ treatments on rice over 3 years in Japan (Kobayashi et al., 1994, 1995). All O₃

1 treatments used CF air, and O₃ was added to the 0.5, 1.0, 1.5, 2.0, or 2.75× ambient
2 concentration for 7 h/day. Based on a linear regression for the 3 years, yield decreased by 3 to
3 10% at a 7-h mean concentration of 40 ppb (Table AX9-16). This decrease is greater than that
4 found for rice in earlier studies in California (Kats et al., 1985), although whether this difference
5 is due to differences in cultivars, experimental treatment, or environmental factors cannot be
6 determined.

7 During 3 years in Beltsville, MD, the soybean cultivars Essex and Forrest were exposed to
8 CF air and NF air in OTCs (Chernikova et al., 2000; Robinson and Britz, 2000) with O₃ added as
9 described for experiments with corn and wheat at Beltsville (Mulchi et al., 1995; Rudorff et al.,
10 1996c). During 1994 and 1995, as previously found for these cultivars, Essex was less sensitive
11 than Forrest, with yield decreases of 10% (n.s.: $p > 0.1$) compared to 32% for Forrest ($p < 0.01$)
12 (Chernikova et al., 2000). There was no evidence of water stress in this experiment. In 1997,
13 the two O₃ treatments were CF (7-h mean = 24 ppb) and NF with a constant addition of O₃ (7-h
14 mean = 58 ppb) (Robinson and Britz, 2000). The yield of Essex was not significantly affected,
15 while the yield of Forrest was decreased by 21% (Table AX9-16).

16 In a study in Raleigh, NC, the soybean cultivar Essex was grown in pots and exposed to CF
17 and 1.5× ambient O₃ concentrations during three growing seasons (Fiscus et al., 1997). Over the
18 3 years, exposure to an average 12-h mean O₃ concentration of 82 ppb reduced soybean yield by
19 41% (Table AX9-16). In similar studies also in Raleigh, NC, Essex was exposed to CF, NF, and
20 1.5× ambient O₃ for two seasons (Heagle et al., 1998). Yield decreased by 16% and 15% in the
21 2 years by ambient O₃ (12-h mean values of 50 and 42 ppb), and decreased by 37 and 40% with
22 exposure to 1.5× ambient O₃ (12-h mean values of 79 and 69 ppb, Table AX9-16). In this same
23 experiment in the second year, similar yield reductions were observed for the cultivar Holladay,
24 while the growth of cultivar NK-6955 was increased substantially by ambient O₃ exposure.
25 All three cultivars were grown in the same chambers in this experiment, and the authors
26 suggested that NK-6955 plants may have shaded the other cultivars to some extent.

27 In a 2-year study using OTCs in Raleigh, NC, the soybean cultivars Coker 6955, Essex,
28 and S53-34 were exposed to CF, NF, and 1.5× ambient O₃ treatments (Miller et al., 1994).
29 Seasonal mean 12-h O₃ concentrations ranged from 14 to 83 ppb. As compared to the CF
30 treatment, ambient O₃ exposure (NF treatment) reduced seed yield by 11 to 18% except for
31 Coker 6955 in the first year (1989), which showed a yield increase of 14%. The 1.5× ambient O₃

1 treatment reduced yield by 32 to 56% in all cultivars in both years. In a similar subsequent
2 experiment with the cultivar Essex, exposure to a 12-h mean ambient O₃ concentration of 50 ppb
3 reduced yield by 11%, while exposure to 79 ppb reduced yield by 22% (Table AX9-16) (Miller
4 et al., 1998). In similar experiments with Essex during 1999 and 2000, plants were exposed to
5 1.5× ambient O₃ in OTCs either in pots or planted in the ground (Booker et al., 2005). Exposure
6 to a 12-h mean ambient O₃ concentration of 75 ppb reduced yield by 27% in pots and 24% in the
7 ground in 1999, and exposure to a 12-h mean ambient O₃ concentration of 67 ppb reduced yield
8 by 41% in pots and 39% in the ground in 2000 (differences between results in pots and in the
9 ground were not statistically significant).

10 These yield reductions for soybean are generally similar to those reported previously in
11 13 similar studies summarized in the 1996 AQCD (Table 5-23 of U.S. Environmental Protection
12 Agency, 1996). A meta-analysis of 53 studies of O₃ effects on soybean found that at an average
13 O₃ exposure of 45 ppb, seed yield was decreased by 10% compared to the CF treatment, while at
14 70 ppb, seed yield was decreased by 24% (Morgan et al., 2003). The 95% confidence limits of
15 these responses based on a bootstrap method did not include a value of zero yield loss. These
16 results suggest that seasonal O₃ concentration patterns that occur in some years throughout many
17 parts of the U.S. can reduce soybean seed yield.

18 A reanalysis of 7 years of data from OTC experiments with wheat in Ostad, Sweden
19 showed that relative yield linearly decreased with increasing O₃, with a maximum yield loss of
20 23% at an AOT40 value of 15 ppm·h (Danielsson et al., 2003). A very similar response was
21 found using the flux (stomatal conductance) model of Emberson et al. (2000b) and a similar
22 amount of the variance was explained by the flux model (for AOT40 model, $r^2 = 0.34$ and for the
23 Emberson flux model, $r^2 = 0.39$). A modified flux model developed and calibrated for this site
24 also had a similar linear response equation, but explained much more of the variance ($r^2 = 0.90$).

25 During the 1990s, a major European research program investigated the combined effects of
26 CO₂, O₃, and other physiological stresses on wheat (Bender et al., 1999; Hertstein et al., 1996,
27 1999; Jäger et al., 1999). The ESPACE-wheat program included 13 experiments in OTCs at
28 eight sites in northern Europe over 3 years. Low- and high-O₃ exposures in these experiments
29 had the following values: 12-h mean (SD) low = 26.3 ppb (12.2), 12-h mean (SD) high = 51.37
30 (18.3) ppb, AOT40 mean (SD) low = 6.2 (8.5) ppm-h, AOT40 mean (SD) high = 28.3 (23.0)
31 ppm-h, as calculated from data presented in Table 3 of Hertstein et al. (1999). An analysis of all

1 13 experiments showed that high O₃ at ambient CO₂ reduced yield by 13% on average (Bender
2 et al., 1999). However, this reduction was not statistically significant based on an ANOVA, and
3 the authors concluded that the wheat cultivar Minaret may be relatively tolerant to O₃ (Bender
4 et al., 1999). Results of some individual studies within this program have been reported
5 previously (Donnelly et al., 1999; Fangmeier et al., 1996, 1999; Mulholland et al., 1997,
6 1998a,b; Pleijel et al., 2000b).

7 In a study with OTCs on silty loam soil in Beltsville, MD, wheat yield was reduced by
8 20% on average over 2 years with 7-h mean concentrations of 61 and 65 ppb O₃, compared with
9 CF treatment with a 7-h mean concentration of 20 ppb (Mulchi et al., 1995; Rudorff et al.,
10 1996a,c). In the above study, different amounts of O₃ were added above ambient levels for 5
11 days as follows: 20, 30, 40, 50, 60 ppb, except that O₃ was not added to exceed a total
12 concentration of 120 ppb (Rudorff et al., 1996c). Wheat grown in pots in OTCs was exposed to
13 elevated O₃ and water stress in Germany, and yield was decreased by 35% in the 2×-ambient
14 treatment with a 7-h mean O₃ concentration of 71 ppb, statistically significant effects were not
15 seen in the 1×-ambient treatment (Fangmeier et al., 1994). In two studies conducted in Raleigh,
16 NC, soft red winter wheat was grown in pots and exposed to CF, NF, and 1.5×-ambient O₃, with
17 ambient and elevated CO₂ concentrations (Table AX9-16) (Heagle et al., 2000). In the first
18 experiment, eight cultivars were exposed to 12-h mean O₃ concentrations of 27, 47, and 90 ppb,
19 and in the second experiment two of these cultivars were exposed to 22, 38, and 74 ppb. There
20 was a trend toward decreased yield in both experiments, but these trends were not statistically
21 significant. The wheat cultivar Drabant was exposed to CF, NF, and a constant addition of 35
22 ppb during 1992 and 1993 in Finland (Ojanpera et al., 1998) using the Heagle-type OTCs
23 (Heagle et al., 1973). The following 12-h mean O₃ exposures were observed in 1992: 14, 30, 61
24 ppb. In 1993, the values were 9, 21, and 45 ppb, (see Table AX9-16 for AOT40 values and other
25 information). Yield was reduced 13% in each year by the added O₃ treatment.

26 The effect of an intermittent constant addition of O₃ using a free air exposure system was
27 investigated with the winter wheat cultivar Riband in Northumberland, UK (Ollerenshaw and
28 Lyons, 1999). Ozone exposures expressed as AOT40 values for September and October 1992
29 were 0.14 and 3.5 ppm·h; while for April to August 1993, values were 3.5 and 6.2 ppm·h. Yield
30 was reduced by 13%.

1 These results provide an additional line of evidence supporting the OTC-studies that
2 demonstrated yield reductions in wheat due to O₃ exposures that occur in the United States.
3 These yield reductions for wheat are generally similar to those reported previously in 22
4 comparable studies summarized in the 1996 AQCD (Table 5-25 of U.S. Environmental
5 Protection Agency, 1996).

6 7 **AX9.5.4.2 Effects on Plant Quality**

8 In addition to reductions in biomass or crop yield, O₃ may also reduce the quality or
9 nutritive value of annual species. Many studies have shown effects of O₃ on various measures of
10 plant organs that affect quality, with most studies focusing on characteristics important for food
11 or fodder (U.S. Environmental Protection Agency, 1996).

12 The effect of a continuous intermittent addition of O₃ using a free air exposure system in
13 Northumberland, UK was investigated with the oilseed rape cultivar Eurol as discussed above
14 (Ollerenshaw et al., 1999). Ozone exposures expressed as AOT40 values for August to October
15 1991 were 0.2 and 3.8 ppm·h; for June 1992 they were 0.7 and 8.1 ppm·h. Yield quality
16 measured as crude protein and oil content was decreased significantly. Because the price of the
17 product is reduced in direct proportion to the oil content, such a decrease represents a substantial
18 loss to growers (Ollerenshaw et al., 1999).

19 Two wheat cultivars, Massey and Saluda, were each grown for one year each in Beltsville,
20 MD (Table AX9-16) and exposed to either CF or an addition of 40 ppb for 7 h/day for
21 5 days/week (Mulchi et al., 1995; Rudorff et al., 1996a,c). Milling and baking quality scores and
22 flour protein were not significantly affected by elevated O₃ exposure, but the softness equivalent
23 was increased slightly (2.4%) in both experiments (Rudorff et al., 1996b). The authors
24 concluded that these changes, along with other slight changes due to an increased CO₂ treatment,
25 suggested that O₃ and CO₂ had only minor effects on wheat grain quality. In wheat grown in
26 Sweden, the harvest index was significantly decreased and the protein content increased due to
27 exposure to a 12-h mean of 48 ppb (Gelang et al., 2000). In an analysis of 16 experiments
28 conducted with spring wheat and either O₃ or CO₂ exposures in four Nordic countries, a negative
29 linear relationship was found between grain yield and grain protein content ($y = -0.38x + 138.6$,
30 expressed as percentages of the NF treatment (Pleijel et al., 1999a).

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

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

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

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

25 26 **AX9.5.4.3 Effects on Foliar Symptoms**

27 For most annual crop species, the most important effects of O₃ are on yield of the
28 commercially important part of the crop, expressed as the mass of the harvested portion.
29 However, for some crops, foliar symptoms are important if they reduce the marketability of the
30 crop. This is why efforts have been made to identify O₃ exposures associated with foliar
31 symptoms. In Europe, Level I critical levels have been determined for such effects based on

1 observations from experiments conducted in 15 countries under the auspices of the United
2 Nations Economic Commission for Europe International Cooperative Programme on effects of
3 air pollution and other stresses on crops and non-woody plants (UN ECE ICP-Vegetation;
4 formerly ICP-Crops), as well as on observations of symptoms in commercial fields from 1993 to
5 1996 (Benton et al., 1995; Benton et al., 2000). Because the occurrence of symptoms increased
6 with greater humidity, these levels took into account the VPD. Two short-term critical levels
7 were derived from 1995 data: an AOT40 value of 0.2 ppm·h over 5 days when mean VPD is
8 below 1.5 KPa (0930 - 1630 h), and a value of 0.5 ppm·h when the mean VPD is above 1.5 Kpa
9 (Benton et al., 1996). The 1996 data supported the critical levels in 83% of observations,
10 although symptoms occurred on three occasions when the AOT40 was less than 0.05 ppm·h and
11 the VPD was very low — less than 0.6 Kpa. The authors concluded that these critical levels are
12 good indicators of the likelihood of foliar symptoms, but that further refinement may be
13 required, such as including factors that modify O₃ uptake by stomata.

14 In a more recent study in Germany, 25 native herbaceous species were exposed to
15 several square-wave O₃ exposures in CF OTCs (Bergmann et al., 1999). Six of the 25 species
16 showed O₃-specific symptoms, and five species responded to single-day peaks. The most
17 sensitive species exhibiting O₃-specific symptoms were *Cirsium arvense* and *Sonchus asper*,
18 which both responded to AOT40 values < 1.5 ppm·h (Bergmann et al., 1999).

19 20 **AX9.5.4.4 Other Effects**

21 Several studies during recent decades have demonstrated O₃ effects on different stages of
22 reproduction. Effects of O₃ have been observed on pollen germination, pollen tube growth,
23 fertilization, and abortion of reproductive structures, as reviewed by Black et al. (2000). This
24 issue is not addressed here, because reproductive effects will culminate for seed-bearing plants
25 in seed production, and the substantial body of evidence relating O₃ exposure and reduced seed
26 production was discussed above. However, one example of a native species will be presented,
27 because of its implications for extrapolating exposure-response data to noncommercial species.
28 Spreading dogbane has been identified as a useful species for O₃ biomonitoring, because
29 of O₃-induced diagnostic symptoms (Kohut et al., 2000). A study in Massachusetts found that
30 exposure to O₃ in NF OTCs or ambient plots for 103 days produced significantly fewer flowers
31 and that fewer of these flowers survived to produce mature fruits (Bergweiler and Manning,

1 1999). Because foliar symptoms were not common, the authors concluded they are not required
2 for effects on reproduction to occur. Genotoxic effects and effects on population genetics were
3 discussed in Section AX9.3.

4 5 **AX9.5.4.5 Scaling Experimental Data to Field Conditions**

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

14 While it is clear that chambers can alter some aspects of plant growth (for example,
15 (Bytnerowicz et al., 2004; Elagöz and Manning, 2005b), the more important issue is whether
16 they alter the response of plants to O₃. A review of such chamber studies done in California
17 found that plants responded similarly to O₃ whether OTCs, closed-top chambers, or air exclusion
18 systems were used; differences were found for fewer than 10% of growth parameters (Olszyk
19 et al., 1986). In another review of literature about Heagle-type OTCs (Heagle et al., 1988), the
20 authors concluded that “Although chamber effects on yield are common, there are no results
21 showing that this will result in a changed yield response to O₃.” A more recent study of chamber
22 effects examined the responses of tolerant and sensitive white clover clones to ambient O₃ in
23 greenhouse, OTCs, and ambient plots (Heagle et al., 1996). For individual harvests, O₃ reduced
24 the forage weight of the sensitive clone 7 to 23% more in the greenhouse than in OTCs.
25 However, the response in OTCs was the same as in ambient plots. A similar study with these
26 white clover clones near Naples, Italy also found no significant difference between O₃ effects
27 measured in OTCs versus those measured by comparing the ratio of sensitive and resistant
28 clones in ambient air (Fagnano et al., 2004). Several studies have shown very similar yield
29 responses to O₃ for plants grown in pots or in the ground, suggesting that even such a significant
30 change in environment does not alter the proportional response to O₃, at least as long as the
31 plants are well- watered (Heagle, 1979; Heagle et al., 1983). As discussed in Section AX9.1,

1 results from recent FACE studies are similar to those obtained from OTC studies, providing
2 another line of evidence that chamber effects in OTCs do not substantially alter O₃ exposure-
3 response relationships.

4 Most experiments investigating O₃ effects on annual vegetation provide adequate water to
5 avoid substantive drought stress. Because drought stress has generally been shown to reduce the
6 effect of O₃ on annual vegetation, such experiments may tend to overestimate O₃ effects on crops
7 and especially on unmanaged or seminatural vegetation.

8 As mentioned above, the use of O₃ flux, rather than exposure, is theoretically more
9 realistic, and such an approach would also address the vertical gradient issue (Section AX9.1).
10 A number of investigators have suggested that modeling O₃ flux can improve estimates of O₃
11 effects on vegetation. Models of O₃ flux can reduce the variation in the response to O₃ that is
12 sometimes observed between years in an experiment (Emberson et al., 2000a, b; Fuhrer et al.,
13 1992; Grünhage et al., 1993; Grünhage and Haenel, 1997; Pleijel et al., 2000a). In a study of O₃
14 deposition to an oat crop in OTCs, O₃ flux in the chamber was estimated to be up to twice that in
15 an adjacent field based on a K-theory approach and measurements of stomatal conductance and
16 environmental conditions (Pleijel et al., 1994b). These measurements were made for 2 hours on
17 5 days when the canopy was physiologically active and wind speeds were moderate. However,
18 the O₃ flux in a plant-less chamber was nearly as high as that in the open field. The authors
19 conclude that O₃ uptake in the chamber was between 100 and 200% of that in the field. These
20 models of flux have a sound biological and meteorological basis and are useful for interpreting
21 experimental data. Flux models have been successfully applied at intensive study sites with
22 detailed site-specific data on stomatal conductance and micrometeorological conditions (e.g.,
23 Fredericksen et al., 1996; Grünhage et al., 1993, 1994)). Yet even at a single well-studied site,
24 different methods can provide different estimates of O₃ flux. For example, at a site in a vineyard
25 in California, an evapotranspiration-based method overestimated the O₃ flux as compared to an
26 eddy covariance approach by 20 to 26% (Massman and Grantz, 1995). At a site in a nearby
27 cotton field the evapotranspiration-based approach overestimated the eddy-covariance method
28 by 8 to 38%. Flux-response relationships have received substantial attention in Europe during
29 the past decade, as part of an attempt to move beyond the exposure-based Level 1 critical levels
30 to flux-based Level II critical levels (Section AX9.4). However, the database for flux-response
31 relationships is very limited (Grünhage et al., 2004), and these approaches do not always explain

1 greater amounts of response variation than do exposure-based approaches (Karlsson et al., 2004).
2 The critical level approach is discussed briefly below, because it has been used to extrapolate
3 from field studies to landscapes, countries, and regions.

4 There has been criticism that the Level I critical level for crops overestimates O₃ effects in
5 the Mediterranean countries, because it was developed based on studies in Northern Europe (De
6 Santis, 1999; De Santis, 2000). However, there is evidence of substantial crop loss due to O₃ in
7 some southern European countries, such as the Po valley in Northern Italy (Fumagalli et al.,
8 2001). In these studies, Heagle-type OTCs were used. Losses in NF chambers as compared to
9 CF chambers over several years at two sites ranged from 11.2 to 22.8% for barley and wheat,
10 from 0.3 to 31.5% for other crop species, and from 4.1 to 19.8% for forage species (Fumagalli
11 et al., 2001). Surprisingly, the least effect was observed for soybean, despite AOT40 values of
12 9.32 ppm·h, 3× the Level I critical level. Similarly, a review of studies in Northern Italy found
13 that ambient O₃ episodes have been reported to cause foliar symptoms on 24 agricultural and
14 horticultural crops in commercial fields (Fumagalli et al., 2001). Ambient O₃ has also been
15 reported to cause yield losses in several crop species, although no data on O₃ exposure were
16 presented by the reviewers (Fumagalli et al., 2001).

17 The Level I approach has also been criticized for focusing only on a single annual species
18 (wheat) and a single woody perennial species (beech). However, this species focus is
19 appropriate, because the goal was to determine an exposure-response relationship for a sensitive
20 species based on available data. In support of standards in Germany, an effort was made to
21 combine data from different species, and consisted of a meta-analysis of studies conducted in
22 both closed and OTCs (Grünhage et al., 2001). In this study, experiments published between
23 1989 and 1999 were included and analyzed if they met the following conditions: (1) a
24 significant O₃ effect was determined; (2) exposure conditions were well defined; (3) foliar
25 symptoms, growth, or yield was measured; and (4) plant species were relevant to Europe
26 (Grünhage et al., 2001). Despite the focus on European species, many of the species studies also
27 occur in the United States. Separate regressions for herbaceous plants and for tree species were
28 created as a function of duration of exposure at a given level of O₃ exposure at the top of the
29 plant canopy. These regression equations, with confidence limits and with correction for the
30 vertical gradient in O₃ from the top of the quasi-laminar boundary layer, can be used to define
31 whether effects are unlikely (below the lower confidence limit), probable (between the

1 confidence limits), or highly likely (above the upper confidence limit) to occur near a given O₃-
2 monitoring station.

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

8 Exposure-response relationships developed primarily from OTC experiments, with
9 confirming evidence from other approaches such as resistant and sensitive clover clones exposed
10 in ambient air and FACE experiments, are useful for estimating the effects of ambient O₃ on
11 vegetation in the U.S. However, despite the substantial number of experimental studies listed in
12 Table AX9-16 and in previous Air Quality Criteria Documents for O₃ (for example, U.S.
13 Environmental Protection Agency, 1996), most studies have been conducted in only a few
14 locations with only a few species of economically important crops. While these studies provide
15 strong evidence that ambient O₃ in the U.S. is likely reducing crop yields and plant growth
16 significantly in many regions in many years, it remains difficult to extrapolate to all landscapes
17 and all plant species and to determine whether exposure-response relationships based on existing
18 studies will protect all species in all locations from significant deleterious effects of O₃.

19 20 **AX9.5.4.6 Summary of Effects on Short-Lived Species**

21 For annual vegetation, the data summarized in Table AX9-16 show a range of growth and
22 yield responses both within species and among species. Nearly all of these data were derived
23 from studies in OTCs, with only two studies using open-air systems in the UK (Ollerenshaw
24 et al., 1999; Ollerenshaw and Lyons, 1999). It is difficult to compare studies that report O₃
25 exposure in different indices such as AOT40, SUM06, or 7-h or 12-h mean values. However,
26 when such comparisons can be made, the results of this more recent body of research confirm
27 the earlier results summarized in the 1996 O₃ AQCD (U.S. Environmental Protection Agency,
28 1996). A summary of earlier literature concluded that a 7-h, 3-month mean of 49 ppb
29 corresponding to a SUM06 exposure of 24.4 ppm·h would cause 10% loss in 50% of 49
30 experimental cases (Tingey et al., 1991). A similar study using a 24-h, rather than 7-h,
31 averaging period found that a SUM06 exposure of 26.4 ppb would cause 10% loss in 50% of

1 54 experimental cases (Lee et al., 1994a;b). Recent data summarized in Table AX9-16 support
2 this conclusion. These values represent ambient exposure patterns that occur in some years over
3 large portions of the United States. Some annual species such as soybean are more sensitive,
4 and greater losses may be expected (Table AX9-16). Thus, the recent scientific literature
5 supports the conclusions of the 1996 AQCD that ambient O₃ concentrations are reducing the
6 yield of major crops in the United States.

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

15 In addition to reductions in crop yield, O₃ may also reduce the quality or nutritive value of
16 annual species. Many studies have shown effects of O₃ on various measures of plant organs that
17 affect quality, with most studies focusing on characteristics important for food or fodder. These
18 studies indicate that there may be economically important effects of ambient O₃ on the quality of
19 crop and forage species. Previous criteria documents have concluded that foliar symptoms on
20 marketable portions of crops and ornamental plants can occur with seasonal 7-h mean O₃
21 exposures of 40 to 100 ppb (U.S. Environmental Protection Agency, 1978, 1986, 1996). The
22 recent scientific literature does not refute this conclusion.

23 The use of OTCs may reverse the usual vertical gradient in O₃ that occurs within a few
24 meters above the ground surface (Section AX9.1). Such a reversal suggests that OTC studies
25 may overestimate, to some degree, the effects of an O₃ concentration measured several meters
26 above the ground. However such considerations do not invalidate the conclusion of the
27 1996 AQCD (U.S. Environmental Protection Agency, 1996) that ambient (Tables AX9-13,
28 Table AX9-21) O₃ concentrations are sufficient to reduce the yield of major crops in the
29 United States.

Table AX9-21. 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

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

Units for SUM06 and W126 are ppm-h.
Source: Baumgardner and Edgerton (1998).

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

2 Although there has been considerable research in Europe on annual species during the past
3 10 years, much research in the United States has focused on perennial species. In Europe, and in
4 a few studies in the United States, effects of O₃ on mixtures of annual and perennial herbaceous
5 species have been investigated using growth chambers, greenhouses, and OTCs. Section
6 AX9.5.5.1 reviews such studies, with an emphasis on studies using OTCs.

7

8 **AX9.5.5.1 Herbaceous Perennial Species**

9 Two alfalfa cultivars were grown in pots and exposed to CF, NF, 1.5×-ambient and
10 2×-ambient O₃ concentrations in two 1-year studies in Quebec, Canada (Renaud et al., 1997).
11 One cultivar, Apica, is commonly grown in the region, and another, Team, is normally grown
12 farther south and is more tolerant to O₃. For Apica in both years and for Team in 1991, O₃
13 exposure caused a linear reduction in above-ground biomass. In the NF treatment, growth of
14 Apica was decreased by 31 and 21% in the 2 years, while the growth of Team was reduced by

1 14% in 1991, but not reduced in 1992. The authors suggested that the differing effects on Team
2 could be due to different progenies and propagation methods in the 2 years or to more rapid
3 growth in 1991 along with higher O₃ peak values in 1991. In 1991, O₃ maxima exceeded 60 ppb
4 in 15 days, whereas in 1992 there were only three such days. At the end of the growing season,
5 total starch reserves in roots were decreased by O₃, due primarily to a decrease in root mass, that
6 the authors suggested could accelerate decline in alfalfa yields. These yield reductions are
7 generally similar to those reported previously in five similar studies summarized in the 1996
8 AQCD (Table 9-25 of U.S. Environmental Protection Agency, 1996).

9 A study in Alabama exposed early- and late-season-planted bahia grass (cultivar
10 Pensacola) in OTCs to CF, NF, or 2×-ambient O₃ treatments (Muntifering et al., 2000). Ozone
11 exposures expressed as 12-h mean values over the 24-week experiment were 22, 45, and 91 ppb,
12 and the highest ambient O₃ concentrations were recorded in late June, late July, late August and
13 mid-September at approximately 90 ppb. Above-ground biomass growth was reduced by the NF
14 treatment for the first and second harvest by 34% and 29% for the early-season planting, but
15 statistically significant effects were not observed in the late-season planting (Table AX9-17).
16 The 2×-ambient treatment did not cause further significant reductions in biomass. The authors
17 suggested that the lack of a significant O₃ effect in the late planting may have been due to the
18 shorter total O₃ exposure time as well as to the lower O₃-exposure concentrations during the
19 weeks immediately preceding harvest. These results are important, because this is an
20 economically important species and because previous studies have focused on grass species that
21 use the C₃, rather than C₄, metabolic pathway.

22 An investigation of the use of different O₃ indices and averaging times on the correlation
23 with growth effects was undertaken with the North Carolina clover system (Heagle et al., 1995).
24 For 2 years of data at six sites in Massachusetts, Oregon, North Carolina, California (2 sites),
25 and Virginia, averaging time was found to be more important than the choice of the type of
26 index including mean, SUM06, and AOT40 (Heagle and Stefanski, 2000). The best correlation
27 between O₃ exposure and the ratio of sensitive-to-tolerant clover types was found for the 6-h
28 period from 1000 to 1600 h. For this period, very similar r² values (0.91 to 0.94) were found for
29 SUM06, W126, and AOT40 (Heagle and Stefanski, 2000). For the above indices, a linear
30 relationship was found, with no effect in Corvallis, OR with exposure to a SUM06 value of

1 10.2 ppm·h and a ratio of 0.53 (sensitive/tolerant) at San Bernardino with a SUM06 exposure of
2 39.4 ppm·h (Heagle and Stefanski, 2000).

3 In a study of the biomass ratio of O₃-sensitive versus O₃-insensitive clover at 14 sites in
4 Europe during 1996 to 1998, a model was developed using ANN techniques (see Section AX9.1)
5 that had r² values for the training data of 0.84 and for unseen validation data of 0.71 (Mills et al.,
6 2000). The predictive factors in the model were AOT40, 24-h mean O₃, daylight mean
7 temperature, and 24-h mean temperature. This model was selected after a thorough investigation
8 of a number of models using many more or fewer parameters using both ANN and multiple
9 linear regression techniques. This model predicted that a 5% reduction in biomass ratio was
10 associated with AOT40 values in the range 0.9 to 1.7 ppm·h accumulated over 28 days, with
11 plants being most sensitive under conditions of low NO_x, moderate temperature, and high 24-h
12 mean O₃ concentration.

13 Exposure to a square-wave 8-h mean O₃ concentration of 92 ppb for 62 days in an
14 experiment in OTCs the UK did not significantly reduce the total yield of strawberry fruits, but
15 did decrease the average size of the fruits by 14% (Drogoudi and Ashmore, 2000). This
16 contrasts with an increase in total yield (fruit weight) found in a previous study in California
17 (Takemoto et al., 1988).

18 When timothy was exposed in OTCs in Sweden to NF, CF, and CF+O₃ treatments, there
19 was no effect of a 12-h mean O₃ exposure of 68 ppb (NF treatment), but a 12-h mean exposure
20 of 152 ppb decreased yield by 58% (Danielsson et al., 1999). A similar lack of effect of
21 exposure to a 12-h mean O₃ exposure of 62 ppb was found in a previous study in the United
22 States (Kohut et al., 1988).

23 Although most investigations of O₃-response relationships focus on growth or yield of
24 marketable portions of plants, some studies also investigate effects on plant quality. In the study
25 of bahia grass in Alabama discussed above, in addition to the effects on yield, there were
26 significant effects on quality for ruminant nutrition (Muntifering et al., 2000). Concentrations of
27 neutral detergent fiber (NDF) were higher in primary-growth and regrowth forages from the
28 early-season planting when exposed to 2×-ambient O₃ than when exposed to the NF treatment.
29 The concentration of acid detergent fiber was higher in the 2×-ambient treatment than in NF
30 treatment regrowth, whereas acid detergent lignin concentration was higher in 2×-ambient than
31 in NF primary-growth forage. Crude protein concentrations were lower in CF-exposed than in

1 NF-exposed regrowth forage from the early planting and in CF- than in NF-exposed primary-
2 growth forage from the initial harvest of the late-season planting. No differences were observed
3 among treatments in concentrations of total phenolics in primary-growth or regrowth forages
4 from either planting, although concentrations of total phenolics tended to be higher in
5 CF-exposed than in NF-exposed primary-growth forage from the late-season planting. The
6 authors concluded that the alterations in quality of primary-growth and vegetative regrowth
7 forages were of sufficient magnitude to have nutritional and possibly economic implications to
8 their use for ruminant animal feed.

9 *Sericea lespedeza* and little bluestem were exposed to CF, NF, and 2×-ambient O₃ in OTCs
10 in Alabama for 10 weeks (Powell et al., 2003). Ozone treatments expressed as 12-h mean
11 concentrations were 23, 40, and 83 ppb and expressed as seasonal SUM06 values were 0.2, 9.1,
12 and 61.0 ppm·h. Although there were few statistically significant effects of O₃ on yield (the
13 yield of only the 2×-ambient compared to NF for *Sericea lespedeza* in the last of six harvests),
14 plant quality as feed for ruminants was reduced. The nutritive quality of *Sericea lespedeza* was
15 decreased by 7% and that of little bluestem by 2% as a result of increased cell wall constituents
16 and decreased in vitro digestibility.

17 For some annual species, particularly crops, the endpoint for an assessment of the risk of
18 O₃ exposure can be defined as yield or growth; e.g., production of grain. For plants grown in
19 mixtures such as hayfields, and natural or seminatural grasslands (including native
20 nonagricultural species), endpoints other than production of biomass may be important. Such
21 endpoints include biodiversity or species composition and measures of plant quality such as total
22 protein and effects may result from competitive interactions among plants in mixed-species
23 communities. Most of the available data on non-crop herbaceous species are for grasslands.

24 In a study of two perennial grasses (bent grass and red fescue) and two forbs (white clover,
25 Germander speedwell) grown in pots in OTCs, O₃ effects differed among species and cutting
26 treatments (Ashmore and Ainsworth, 1995) (see also Table AX9-17). Fescue biomass increased
27 with higher O₃ treatments both in pots that were not cut during the growing season (mid-June to
28 mid-September) and those that were cut every two weeks. However, bent grass biomass
29 decreased with higher O₃ exposure in the uncut treatment and increased in the cut treatment.
30 White clover and Germander speedwell biomass decreased substantially with higher O₃ exposure
31 with and without cutting, with greater decreases in the cut treatment. The authors cautioned that

1 the experiment did not replicate field circumstances. The plants were all cut to 1 cm above the
2 ground, which does not simulate grazing, and there may have been effects due to growing the
3 plants in pots. However, two key results of this study likely apply to mixtures of species
4 growing in hay or forage fields or seminatural and natural communities. First, O₃ exposure
5 increased the growth of O₃-tolerant species, while exacerbating the growth decrease of O₃
6 sensitive species. Second, the total biomass of the mixed-species community was unaffected by
7 O₃ exposure due to the differential effects on O₃-sensitive and O₃-tolerant species.

8 In a 2-year study using OTCs placed over managed pasture in Switzerland, the above-
9 ground biomass of clover (red and white) was reduced linearly in response to increased O₃
10 exposure (Table AX9-17) (Fuhrer et al., 1994). Exposure to a 12-h mean concentration of
11 39 ppb O₃ reduced biomass by 24% as compared to the CF treatment with a 12-h mean
12 concentration of 21 ppb O₃. There was a trend toward increased above-ground biomass of
13 grasses (primarily orchard grass), but this trend was not statistically significant. As often found
14 in other studies of mixtures of species, by O₃ exposure did not significantly affect total above-
15 ground biomass O₃ exposure.

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

26 A mixture of species in an old farm field in Alabama was exposed to O₃ for two growing
27 seasons in large OTCs (4.8 m high and 4.5 m diameter); and a similar lack of effect of O₃ was
28 found on total plant community growth measured as both canopy cover and vertical canopy
29 density (Barbo et al., 1998). Of the 40 species in the plots, O₃ effects were examined only on the
30 most common species: blackberry, broomsedge bluestem, bahia grass, *Panicum* spp., and
31 winged sumac (second year only). Of these species, a 2×-ambient O₃ treatment increased the

1 percent canopy cover of blackberry over 2 years by 124%, while that of winged sumac was
2 decreased by 95% (Table AX9-17). Blackberry showed no significant effect on biomass, but
3 ripe fruit mass was decreased by 28% (Chappelka, 2002). However, there was a significant
4 chamber effect for this latter response. Biomass was not reported for other species in this study
5 due to a hurricane. Effects on loblolly pine grown in this experiment are discussed subsequently
6 in Section AX9.5.5.5.

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

21 22 **AX9.5.5.2 Deciduous Woody Species**

23 It is extremely difficult and costly to study entire mature trees under controlled conditions
24 such as those in OTCs, with the possible exception of some species managed for fruit or nut
25 production. For this reason, the great majority of investigations have been of seedlings in
26 growth chambers, greenhouses, or OTCs. A few investigations have been carried out on
27 saplings or more mature trees using free air exposure systems (Haeberle et al., 1999; Isebrands
28 et al., 2000, 2001; Werner and Fabian, 2002). Exposure-response functions based on 28
29 experimental cases of seedling response to O₃ suggest that a SUM06 exposure for 3 months of
30 31.5 ppm·h would protect hardwoods from a 10% growth loss in 50% of the cases (Table
31 AX9-18). However, there is a substantial range in sensitivity among species. A risk analysis was

1 undertaken to predict tree biomass growth reductions due to O₃ based on exposure-response
2 equations for seedlings of individual species combined with the species' spatial distribution
3 across the eastern United States and interpolated O₃ exposure expressed as SUM06 (Hogsett
4 et al., 1997). The growth of sensitive species such as aspen and black cherry was predicted to be
5 reduced by at least 20% across 50% of their ranges in a high O₃ year and approximately 10% in a
6 lower-than-average O₃ year (Hogsett et al., 1997).

7 A few investigations reported since the last criteria document was prepared have examined
8 saplings or mature trees, notably of oak species in the southern Appalachian Mountains and pine
9 species in California. Most of these studies have been of natural (uncontrolled) O₃ exposures.
10 Additional studies have examined foliar symptoms on mature trees, and in recent years such
11 surveys have become more common and with greater attention to the standardization of methods
12 and the use of reliable indicator species (Campbell et al., 2000; Smith et al., 2003). Previous
13 criteria documents have noted the difficulty in relating foliar symptoms to effects on individual
14 tree growth, stand growth, or ecosystem characteristics (U.S. Environmental Protection Agency,
15 1996). This difficulty still remains to the present day.

16 Some investigators have suggested that a comprehensive risk assessment of the effects of
17 O₃ on mature tree species might best be accomplished by extrapolating measured effects of O₃
18 on seedlings to effects on forests using models based on tree physiology and forest stand
19 dynamics (Chappelka and Samuelson, 1998; Laurence et al., 2000, 2001). Several such efforts
20 are discussed in Sections AX9.3 and AX9.6.

21 In this subsection, emphasis will be placed on experimental evidence of O₃ effects on the
22 growth of woody species under controlled conditions with some information from observational
23 studies under ambient conditions in forests. Experimental results are summarized for deciduous
24 species in Table AX9-18; the species are discussed below in the order in which they appear in
25 this table.

26 A series of studies in Michigan and Wisconsin during the 1990s on clones of trembling
27 aspen previously demonstrated that they differ in their O₃ sensitivity (Coleman et al., 1995a,b,
28 1996; Dickson et al., 2001; Isebrands et al., 2000, 2001; Karnosky et al., 1996, 1998, 1999; King
29 et al., 2001). Several of those studies were undertaken with plants in pots or in the ground in
30 OTCs and additional studies were undertaken at three sites selected to differ primarily in O₃
31 exposure (Karnosky et al., 1999). An ongoing study was undertaken using a FACE carbon

1 dioxide and O₃ enrichment facility in Rhinelander, WI (Isebrands et al., 2000, 2001). These
2 studies showed that O₃-symptom expression was generally similar in OTCs, FACE, and gradient
3 sites, supporting the previously observed variation among aspen clones (Karnosky et al., 1999).
4 In the Michigan OTC study, plants were grown in pots and exposed to CF, 0.5× ambient, 1×
5 ambient, 1.5× ambient, 2×-ambient O₃ treatments for 98 days (Karnosky et al., 1996). Ozone
6 concentrations expressed as 3-month, 7-h mean values were 7 ppb (CF), 43 ppb (1×) and 63 ppb
7 (2×). Ozone decreased total plant biomass between 2 and 22% for three clones previously
8 selected to represent high, intermediate and low O₃ tolerance based on previous studies of larger
9 populations (clones 216, 271 and 259; Karnosky et al., 1996). Seedlings produced from 15 parent
10 trees responded similarly as did the 3 clones, with an average biomass reduction of 14% in the
11 1×-ambient treatment for the seedlings compared to 16% for the clones. In a second experiment
12 using square wave exposures, biomass reduction for the clones was ranged from 23 to 39%
13 (mean = 31%) at a 7-h mean O₃ concentration of only 45 ppb, which is similar to the response to
14 the 2×-ambient treatment in the previous experiment at a 7-h mean O₃ concentration of 66 ppb.

15 The FACE study evaluated the effects of multiple years of exposure to combinations of
16 elevated CO₂ and 1.5× ambient O₃ on growth responses in mixture of five trembling aspen
17 clones (Isebrands et al., 2000, 2001). Height, diameter, and stem volume (diameter² × height)
18 were decreased by elevated O₃. On average for all clones, stem volume was decreased by 20%
19 over the first 3 years in the elevated O₃ treatment as compared with the 1×-ambient treatment.
20 However, one clone showed increased growth in response to O₃. Ozone concentrations were not
21 reported. Over the first 7 years of the study, average stem volume was decreased by 14% with
22 12-h mean O₃ concentrations between 49 and 55 ppb as compared with effects at ambient O₃
23 concentrations with 12-h mean values of 35 to 37 ppb (O₃ exposure data are for the first 4 years,
24 as they have not been reported for subsequent years) (Karnosky et al., 2003, 2005). This FACE
25 facility study is important, because it confirmed responses reported previously with these clones
26 grown in pots or soil in OTCs, without the alterations of microclimate induced by chambers.
27 Currently, this is the only U.S. study using this technology to have examined the effects of O₃
28 under these conditions. This study is also significant, because the elevated O₃-exposure pattern
29 used was intended to reproduce the 6-year average pattern from Washtenaw County, Michigan
30 (Karnosky et al., 1999).

1 Rooted cuttings of two aspen clones from Acadia National Park in Maine were exposed to
2 1×-ambient, 1.7×-ambient, and 3×-ambient O₃ concentrations in large OTCs in Ithaca, NY for
3 much of one growing season (15 June to 15 September) (Yun and Laurence, 1999). Both
4 circular (4.7 m diameter, 3.7 m height) and rectangular (7.4 m × 2.75 m × 3.7 m height)
5 chambers were used (Mandl et al., 1989). Exposure to 1.7×-ambient O₃ (SUM06 = 20 ppm·h,
6 9-h mean = 74 ppb) reduced shoot growth by 14 and 25% compared to ambient O₃ for the two
7 clones (Yun and Laurence, 1999). Total dry weight was reduced by 55 and 35% in the two
8 clones by the 3×-ambient treatment (SUM06 = 62 ppm·h, 9-h mean = 124 ppb) compared to the
9 ambient O₃ treatment.

10 When black poplar cuttings in OTCs in Belgium were exposed to 8-h mean O₃
11 concentrations of 5, 29, and 33 ppb, diameter growth decreased by 29% in the highest O₃
12 treatment, but height growth was unaffected (Bortier et al., 2000b). A 2-month study of hybrid
13 poplar (*Populus tremuloides* × *P. tremula*) in a free air exposure system in Finland with 7-h
14 mean O₃ concentrations of 30 and 38 ppb found a 6% decrease in height with no effect on
15 biomass (Oksanen et al., 2001). Eastern cottonwood cuttings in pots buried in the ground with
16 drip irrigation were exposed to ambient O₃ at several sites in and near New York City in three
17 2-month experiments during three summers (Gregg et al., 2003). Ozone concentrations were
18 lower at urban sites than at rural sites within 100 km of the urban sites. Total biomass growth
19 was greater in urban than rural sites, with a strong linear decrease in biomass with increasing O₃
20 across all sites and years ($r^2 = 93$). Total biomass decreased 33% with 12-h mean O₃ levels of 38
21 ppb compared to 23 ppb. Multiple regression analysis showed no significant temperature effect
22 on biomass. Therefore, the authors suggested that O₃ exposures were the most likely explanation
23 for the reduced biomass in rural areas. The overall growth reductions and the variation among
24 genotypes seen on all of the above aspen and poplar studies is similar to those previously
25 reported in three OTCs studies summarized in the 1996 O₃ AQCD (Table 9-26 of U.S.
26 Environmental Protection Agency, 1996).

27 For paper birch, over the first 7 years of the Wisconsin FACE study, average stem volume
28 was unaffected by 12-h mean O₃ concentrations between 49 and 55 ppb as compared with effects
29 at ambient O₃ concentrations with 12-h mean values of 35 to 37 ppb (O₃ exposure data are for
30 the first 4 years, as they have not been reported for subsequent years (Karnosky et al., 2003,
31 2005). In contrast, significant effects were found in this study for aspen and sugar maple, so

1 these results indicate that paper birch is relatively insensitive to O₃ compared to these other
2 species.

3 Black cherry seedlings grown in pots were exposed in OTCs in the Great Smoky Mountain
4 National Park in Tennessee to O₃ treatments ranging from CF to 2×-ambient in two experiments
5 during 1989 and 1992 (Neufeld et al., 1995). Ozone exposure, expressed as SUM06, ranged
6 from 0 to 40.6 ppm·h in 1989, and from 0 to 53.7 ppm·h in 1992. Corresponding AOT40 values
7 were 0.0 to 28.3 ppm·h in 1989, and 0 to 40.4 ppm·h in 1992. In 1989, total biomass was
8 decreased in the 1.5×-ambient treatment by 18% and in the 2×-ambient treatment by 38%.
9 In 1992, total biomass was decreased in the 1.5×-ambient treatment by 27%, and in the
10 2×-ambient treatment by 59%. In this study, SUM06 and AOT40 provided better fits than did
11 SUM00 with Weibull regression analyses to log-transformed biomass data. Although a Weibull
12 model was used, responses to O₃ expressed as SUM06 and AOT40 appeared to be linear. The O₃
13 exposures in the 1.5×-ambient and 2×-ambient treatments were reported to be similar to those
14 for a site near Charlotte, NC in a high-O₃ year (1988). In a 2-year experiment in OTCs in Ohio,
15 seedlings of black cherry, sugar maple, and yellow poplar were exposed to O₃ treatments with
16 SUM00 values ranging from 16 to 107 ppm·h in 1990 and 31 to 197 ppm·h in 1991 (Rebeck,
17 1996). After two seasons of exposure, only black cherry showed a growth decrease: total
18 biomass was reduced by 32% in the 2×-ambient O₃ treatment compared to the CF treatment; root
19 biomass was decreased by 39%. These results contrast with those of a previous study with black
20 cherry seedlings in which significant biomass reductions with exposures up to 2×-ambient were
21 not observed (7-h mean = 21 to 97 ppb), perhaps because of the small sample size (3 seedlings
22 per chamber (Samuelson, 1994) in the earlier study.

23 A multiyear study of effects of O₃ on both seedling and mature (30-year-old) red oak trees
24 was conducted in Norris, TN in large OTCs with three replicates per O₃ treatment. Trees were
25 exposed for 3 years to CF, 1×-ambient, and 2×-ambient treatments, with the following O₃
26 exposures: SUM06 for 3 years = 0, 29, 326 ppm·h; SUM00 for 3 years = 147, 255, and 507
27 ppm·h. The net photosynthetic rate in mature trees was reduced by 25% in the ambient
28 treatment and by 50% in the 2×-ambient treatment (Samuelson and Edwards, 1993; Hanson
29 et al., 1994; Wullschleger et al., 1996). Despite these large decreases, no significant effects on
30 stem increment at the base, stem increment in the canopy, or leaf mass were observed for the
31 mature trees (Samuelson et al., 1996). Similarly, seedling biomass was not significantly reduced

1 by O₃ exposure. The difficulty in replicating experiments with mature trees makes it difficult to
2 detect changes in growth or biomass. However, the mean values of the stem increment at the
3 base and within the canopy in the ambient treatment were larger than those in the CF treatment,
4 although those in the 2×-ambient treatment were lower. Therefore, this study of mature trees
5 does not provide evidence that these ambient concentrations reduced above-ground tree growth,
6 even after 4-years exposure.

7 Sugar maple seedlings were exposed for 3 years to ambient, 1.7×-ambient, and 3×-ambient
8 O₃ treatments at both high-light (35% of ambient) and low-light levels (15% of ambient)
9 (Topa et al., 2001). This experiment was conducted in large OTCs near Ithaca, NY. Over the
10 3 years, O₃ exposures expressed as SUM00 for the three treatments were 88, 126, and 185
11 ppm·h. After 3 years, total seedling biomass in the 3×-ambient treatment was reduced by 64%
12 and 41% in the low- and high-light treatments, respectively (compared to the 1×-ambient
13 treatment). The larger reduction of biomass under low-light conditions suggests that seedlings
14 growing under closed canopies may be substantially more sensitive to O₃ than are seedlings
15 exposed to higher-light levels in gaps or clearings. These results differ from other studies in
16 which seedling biomass was unaffected by exposure to SUM00 values of 304 ppm·h over 2 years
17 (Rebbeck, 1996) or 591 ppm·h over 3 years (daytime mean of 40.7) (Laurence et al., 1996).
18 However the latter two studies used much higher light levels, which may have reduced the O₃
19 effect, based on the results of Topa et al. (2001). Over the first 7 years of the Wisconsin FACE
20 study, average stem volume of sugar maple was decreased by 14% with 12-h mean O₃
21 concentrations between 49 and 55 ppb as compared with effects at ambient O₃ concentrations
22 with 12-h mean values of 35 to 37 ppb (O₃ exposure data are for the first 4 years as they have not
23 been reported for subsequent years (Karnosky et al., 2003, 2005). These growth effects were not
24 statistically significant for the first 3 years, but became significant subsequently. These results
25 are important because they demonstrate that 3 years of exposure may not be long enough to
26 evaluate effects of O₃ on the growth of tree species.

27 Although most studies demonstrate that O₃ decreases biomass growth, occasional results
28 indicate that O₃ can increase growth of some portions of woody perennials. When Casselman
29 plum trees near Fresno, CA were exposed to O₃ in large, rectangular OTCs to three O₃ treatments
30 (CF, 1×-ambient, and an above-ambient O₃ treatment) for 4 years (12-h mean = 31, 48, 91
31 ppm·h), stem increment increased 14% in the highest O₃ treatment compared to the CF

1 treatment; and this effect was statistically significant (Retzlaff et al., 1997). However, fruit yield
2 decreased in this treatment by 42% and also decreased by 16% in the 1×-ambient-O₃ treatment.
3 Root growth was not measured in this study. Hence, the increase in stem diameter may have
4 been at the expense of other organs. However, in a fifth year, all plants were exposed to
5 1×-ambient O₃, and there were no differences in fruit yield, suggesting that trees were able to
6 recover to some extent from the effects of O₃ exposure in prior years.

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

12 Many studies have demonstrated that root growth is more sensitive to O₃ exposure than is
13 stem growth. For example, in a study with black cherry seedlings exposed in OTCs in
14 Tennessee in 1989, root biomass in a 2×-ambient treatment was decreased by 42%, while stem
15 biomass was decreased by only 24%. However, in a second experiment in 1992, root and stem
16 growth reductions in the 2×-ambient treatment were similar (65% versus 60%) (Neufeld et al.,
17 1995). In Finland, reduced root growth was found for a number of clones of silver birch
18 (Oksanen and Saleem, 1999). After 5 years, root growth was decreased by 33%, but shoot
19 growth was not affected by O₃ exposures of a 7-h mean of 15 ppm·h over 5 years in a FACE
20 system (Oksanen, 2001). When first-year poplar seedlings (*P. tremuloides*) were exposed in
21 OTCs to two O₃ concentrations and six N concentrations, the root/shoot ratio was decreased soon
22 after exposure to O₃ in all N treatments, even though O₃ effects on total biomass were not
23 detected in the low-N and very high-N treatments (Pell et al., 1995). These results suggest that
24 effects on the root/shoot ratio occur before significant growth effects arise. In a series of OTC
25 experiments lasting 1 to 3 years at 3 different elevations in Switzerland, fine root growth in
26 European beech was found to be more sensitive to O₃ than was shoot or total biomass (Braun and
27 Fluckiger, 1995). Although the estimated effect of O₃ on fine root biomass was similar to that
28 for total biomass, fine root biomass was significantly decreased at AOT40 (24-h) values of only
29 3 ppm·h, while total biomass was not significantly decreased until AOT40 values reached 30 to
30 40 ppm·h.

1 **AX9.5.5.3 European Critical Levels**

2 In Europe, a Level I critical level has been set for forest trees based on OTC studies of
3 saplings. This level is discussed here because it was based on a deciduous tree species. For
4 consistency with the approach used for crops, an AOT40 index value was selected. A few
5 studies have shown that O₃ can be taken up by tree species at nighttime, e.g., young birch trees
6 (Matyssek et al., 1995). However, because most evidence suggests that O₃ deposition at
7 nighttime is low (Coe et al., 1995; Rondon et al., 1993), a value for only daylight hours was
8 selected in Europe (Fuhrer et al., 1997; Kärenlampi and Skärby, 1996). European beech was
9 selected for development of a Level I critical level, because data from several studies were
10 available for this species and because deciduous tree species were judged to be more sensitive to
11 O₃ compared to evergreen tree species (Fuhrer et al., 1997; Kärenlampi and Skärby, 1996).
12 A critical level was defined as an AOT40 value of 10 ppm·h for daylight hours for a 6-month
13 growing season (Kärenlampi and Skärby, 1996). However, other studies have shown that other
14 species such as silver birch may be more sensitive to O₃ than beech (Pääkkönen et al., 1996).
15 Level I critical values are not designed for making quantitative estimates of the O₃ effects on
16 vegetation at the regional scale, instead a so-called Level II critical value is required for this
17 purpose. For long-lived perennials, additional problems complicate extrapolation. As discussed
18 below (Section AX9.5.5.7), considerable scaling is required to extrapolate from experiments
19 conducted with tree seedlings to estimate effects on mature trees in forests. Because of these
20 scaling issues, there is greater uncertainty in estimating effects on forest trees than on annual
21 plants such as crops. While some information is available for addressing issues such as scaling
22 from seedlings to mature trees and estimating O₃ uptake, this information may still be
23 insufficient for developing a Level II approach that can provide quantitative estimates of forest
24 growth losses due to O₃ (Broadmeadow, 1998).

25 26 **AX9.5.5.4 Summary of Effects on Deciduous Woody Species**

27 Recent evidence from free air exposure systems and OTCs supports results observed
28 previously in OTC studies (Table AX9-15, Figure AX9-18). Specifically, a series of FACE
29 studies undertaken in Rhinelander, WI (Isebrands et al., 2000, 2001) showed that O₃-symptom
30 expression was generally similar in OTCs, FACE, and ambient-O₃ gradient sites, supporting the
31 previously observed variation among aspen clones using OTCs (Karnosky et al., 1999). This

1 study also found no effects on sugar maple growth after 3 years, but in years 4 to 7 found
2 significant growth reduction due to O₃ (Karnosky et al., 2005). These results are important,
3 because they indicate results obtained from OTCs are supported by results from free air exposure
4 systems and also that more than 3 years may be required to adequately investigate the effects of
5 O₃ on the growth of tree species. Finally, this study found that competition may alter the effect
6 of O₃, depending on environmental conditions and genotype (McDonald et al., 2002). New
7 evidence is also available comparing various aspects of O₃ sensitivity between seedlings and
8 mature trees of some species, notably red oak. As has been observed in previous criteria
9 documents, root growth is often found to be the most sensitive indicator in terms of biomass
10 response to O₃.

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

27 28 **AX9.5.5.5 Evergreen Woody Species**

29 Most investigations have shown evergreen tree species to be less sensitive to O₃ compared
30 to deciduous species (U.S. Environmental Protection Agency, 1996). For example, exposure-
31 response functions based on 23 experimental cases of seedling response to O₃, suggest that a

1 SUM06 exposure for 3 months of 42.6 ppm·h would protect evergreen species from a 10%
2 growth loss in 50% of the cases (Table AX9-15). For deciduous species, the corresponding
3 SUM06 value was 31.5 ppm·h (Table AX9-15). As another example, experiments in the Great
4 Smoky Mountains National Park found black cherry seedlings to demonstrate substantial
5 decreases in biomass, as discussed above and shown in Table AX9-18 (Neufeld et al., 1995).
6 However, exposure for up to three growing seasons did not decrease the biomass of eastern
7 hemlock, Table Mountain pine, and Virginia pine seedlings exposed to O₃ under similar
8 conditions in this location, as shown in Table AX9-19 (Neufeld et al., 2000).

9 As for deciduous species, there is a substantial range in sensitivity among evergreen
10 species. As discussed above for deciduous species, a risk analysis was undertaken to predict tree
11 biomass growth reductions due to O₃ based on exposure-response equations for tree seedlings
12 combined with species distribution across the eastern United States and interpolated O₃ exposure
13 (Hogsett et al., 1997). While some species such as Virginia pine were predicted to be affected
14 only slightly even in a high O₃ year, the growth of sensitive evergreen species such white pine
15 was predicted to be reduced by 5% in a lower-than-average O₃ year and 10% in a high O₃ year
16 across 50% of its range (Andersen et al., 1997). The remainder of this section discusses
17 experimental results for evergreen species in the order shown in Table AX9-19.

18 Douglas fir seedlings were exposed to elevated O₃ concentrations in a free air zonal air
19 pollution system in British Columbia, Canada for two growing seasons with 12-h mean values in
20 1988 of 18 to 41 ppb and in 1989 of 27 to 66 ppb (Runeckles and Wright, 1996). Although
21 substantial variation was seen in effects among the different treatments, there was a significant
22 decrease in the growth of the second flush weight in the second year, with reductions of 55% at
23 the highest O₃ exposure, based on a linear regression. This result contrasts with the lack of
24 effect seen in a previous study with seedlings of this species grown in pots for 134 days and
25 exposed to 7-h mean O₃ concentrations up to 71 ppb (Table 9-30 in U.S. Environmental
26 Protection Agency, 1996).

27 First-year loblolly pine seedlings of 53 open-pollinated families were exposed to
28 1×-ambient O₃ in OTCs for a single growing season, and average growth volume was decreased
29 by 14% compared to a CF treatment (McLaughlin et al., 1994). The 1×-ambient O₃ exposure in
30 this study expressed as 24-h SUM00 was 137 ppm·h, and the CF treatment reduced O₃ by 47%.
31 In this study, the root-to-shoot ratio was decreased significantly in six of the nine families

1 examined. Exposure to O₃ with SUM06 values up to 162 ppm·h and 132 ppm·h in 2 successive
2 years in OTCs had no effect on seedlings grown in competition with various species of grasses
3 and forbs (Barbo et al., 2002). Exposure of 3-year-old seedlings to O₃ exposures of up to
4 2.5×-ambient (12-h mean of 98 ppb) also had no significant effect (Anttonen et al., 1996).
5 Four-week-old loblolly pine seedlings were grown in large OTCs in Alabama and exposed to
6 CF, 1× ambient, 2× ambient O₃ treatments in 2 one year experiments, with seasonal 12-h
7 mean O₃ concentrations of 13, 47, 98 ppm·h in 1998 and 12, 44, 97 ppm·h in 1999 (Estes et al.,
8 2004). Shoot biomass was decreased 15% in the 1× treatment and 22% in 2× in both years, while
9 root biomass was decreased by 26% in the 2× treatment in both years. Foliar symptoms were
10 significantly greater in the 1× treatment in 1999 and in the 2× treatment in both years.
11 Information summarized in the 1996 AQCD (U.S. Environmental Protection Agency, 1996),
12 indicated that significant effects on seedling growth were observed in several studies of
13 seedlings exposed to elevated O₃ concentrations for one or more years. Several studies,
14 including that of McLaughlin et al. (1994), demonstrate considerable variation in O₃ sensitivity
15 among different genotypes of loblolly pine.

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

27 For several tree species, O₃ has been shown in experimental studies with seedlings to
28 reduce root growth more than shoot growth (U.S. Environmental Protection Agency, 1996).
29 Ponderosa pine has been shown to be sensitive to O₃, and studies with seedlings have shown
30 reduced root growth, decreases root-to-shoot ratios, and decreased allocation to roots (Andersen
31 et al., 1991, 1997; Andersen and Rygiewicz, 1995; Andersen and Scagel, 1997; Tingey et al.,

1 1976). Data from a long-studied pollution gradient in the San Bernardino Mountains of southern
2 California suggests that O₃ substantially reduces root growth in natural stands of ponderosa pine.
3 Root growth in mature trees was decreased at least 87% in a high pollution site as compared to a
4 low pollution site (Grulke et al., 1998), and a similar pattern was found in a separate study with
5 whole tree harvest along this gradient (Grulke and Balduman, 1999). Because other potential
6 influences on root growth, including shading by competing trees, soil temperature, soil moisture,
7 phenology, were not correlated with the observed pattern of reduced root growth, the authors
8 conclude that O₃ was the cause of the observed decline in root growth. Further results of field
9 investigations with ponderosa pine and other pine species native to California are discussed
10 below under the heading “Scaling experimental data on long-lived species to field conditions” as
11 well as in Section AX9.6.

12 Table Mountain pine, Virginia pine, and eastern hemlock seedlings were exposed to
13 various levels of O₃ (CF to 2× ambient) in OTCs for in a series of experiments two or three years
14 in Great Smoky Mountains National Park in Tennessee (Neufeld et al., 2000). There were no
15 statistically significant effects of O₃ exposure on stem or root biomass, and only slight effects on
16 the biomass of the oldest needles in Table Mountain pine in the 2× ambient treatment.

17 As reviewed in the 1996 criteria document, studies of the response of red spruce to O₃
18 exposures generally have not found effects on growth of seedlings or saplings, even after
19 exposure to high concentrations (12-h mean of 90 ppb) for up to 4 years. A report since that
20 time confirms that this slow-growing species is O₃ insensitive for at least several years (Laurence
21 et al., 1997).

22 For perennial vegetation, cumulative effects over more than one growing season may be
23 important. For 3-year-old Norway spruce in Sweden, exposure to elevated O₃ for three growing
24 seasons decreased total biomass by 18% and stem biomass by 28% (Karlsson et al., 1995).
25 However, after a fourth season of exposure, total biomass decreased significantly by only 8%
26 (Karlsson et al., 2002). In this experiment, the O₃ exposures expressed as 12-h mean values
27 averaged over four growing seasons were 12 and 44 ppb for the CF and 1.5×-ambient treatments,
28 respectively; and AOT40 values were 2 and 23 ppm·h, respectively. Despite 4 years of
29 exposure, this experiment did not demonstrate a consistent trend in the O₃ effect on biomass that
30 would suggest a significant carry-over effect. However, a study of 3- to 7-year-old Norway

1 spruce in OTCs in Finland found a 5.3% decrease in total plant biomass after 7 years, with an
2 elevated O₃ AOT40 exposure value of 79 ppm•h (Ottoosson et al., 2003; Wallin et al., 2002).

3 4 **AX9.5.5.6 Summary of Effects on Evergreen Woody Species**

5 In summary, the O₃ sensitivity of different genotypes within species and between species
6 of evergreen vegetation varies widely. Based on studies with evergreen seedlings in OTCs,
7 major species in the United States are generally less sensitive than are most deciduous trees, and
8 slower-growing species are less sensitive than faster-growing ones. However, exposure to
9 ambient O₃ may reduce the growth of seedlings of commonly occurring species. Because tree
10 species are long-lived, most experiments have only covered a very small portion of the life span
11 of a tree, making estimation of any effect on mature trees difficult. Considerations for scaling
12 the results of seedling studies to mature forest trees as well as additional information from field
13 surveys and studies of mature trees under natural conditions are discussed below and in
14 Section AX9.6.

15 16 **AX9.5.5.7 Scaling Experimental Data to Mature Trees**

17 As compared with annual crop species, it is much more difficult to define appropriate
18 exposure-response relationships for tree species. For annual species, an experiment may cover
19 the whole life span of the plant, but it is difficult and expensive to provide controlled-exposure
20 conditions for long-lived plants for any significant portion of their life spans, although a few
21 FACE studies have demonstrated that it is feasible. However, FACE studies cannot investigate
22 the effects of ambient O₃ exposures, because lower-than-ambient O₃ treatments cannot be
23 applied. Most studies have used small seedlings, because they are manageable under
24 experimental conditions; but seedlings and mature trees may have different sensitivities to O₃.
25 For perennial species, effects of O₃ may accumulate over more than 1 year, and may interact
26 with other stresses such as drought stress over multiple growing seasons. As for annual species
27 (Section AX9.3.2), substantial variability occurs among evergreen genotypes and this variation
28 may interact with other stress responses differently in different landscapes and regions. Despite
29 these difficulties, investigators have addressed some of these issues since the 1996 AQCD (U.S.
30 Environmental Protection Agency, 1996). New information is available on the response of
31 mature evergreen trees to O₃ under field conditions, and models based on tree physiology and

1 stand dynamics have been used to predict O₃ effects on forest stands and regions. The following
2 issues are reviewed briefly below: (1) interaction of drought and O₃ stress, (2) scaling data from
3 seedlings to mature-tree studies. Two additional scaling issues are addressed in Section AX9.6:
4 (1) scaling data to forest stands, and (2) scaling data to ecosystems and regions.

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

7 Many interacting factors may influence the effect of O₃ on vegetation. For crop plants,
8 environmental conditions are often managed such that nutrients and water are not strongly
9 limiting; but for native vegetation, including most perennial species, such factors are likely to
10 limit growth. The effects of interacting stresses on vegetation were reviewed in Section AX9.3.
11 However, because drought is common in many forests, and because there is substantial evidence
12 that it alters the response of trees to O₃, it is discussed in this section in the context of
13 determining exposure-response relationships for trees.

14 Controlled experiments with seedlings provide direct evidence that drought can reduce the
15 impact of O₃. For example, for 3-year-old Norway spruce in Sweden, exposure to elevated O₃
16 for three growing seasons decreased total biomass by 18% and stem biomass by 28% (Karlsson
17 et al., 1995). However, for droughted trees, both total and stem biomass decreased only 5%,
18 with a statistically significant interaction with O₃ for stem biomass. Yet after a fourth season of
19 exposure, there was no longer any interaction between drought and O₃, while there was a
20 significant decrease of 8% in the biomass when both drought and well-watered data were
21 combined (Karlsson et al., 2002). In this study, seedlings were grown in sand in 120-L pots and
22 for the drought treatment, water was withheld for 4 weeks during the first year and for 7 to 8
23 weeks during each of the last 3 years. In this experiment, the O₃ exposures expressed as 12-h
24 seasonal daylight mean averaged over four growing seasons were 12 and 44 ppb for the CF and
25 1.5×-ambient treatments, respectively. Over this period, the AOT40 values for the treatments
26 averaged 2 and 23 ppm·h respectively. Despite 4 years of exposures, this experiment did not
27 demonstrate a consistent trend in drought O₃ interactions. The difference in effects seen between
28 the third and fourth season suggest that scaling drought-O₃ interactions from seedlings to mature
29 trees may be difficult. However, evidence from biomonitoring surveys supports an interaction
30 between drought and O₃ effects, at least for foliar symptoms. In systematic surveys of foliar
31 symptoms on species selected as biomonitors throughout much of the eastern United States,

1 symptoms were more common and more severe in areas with high O₃ concentrations (Smith
2 et al., 2003). However, in very dry years, such as 1999, the occurrence and severity of
3 symptoms was greatly reduced, even in areas with high ambient O₃ concentrations.
4

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

6 Because most experiments are conducted with seedlings, various methods are required to
7 scale experimental data on seedlings to mature trees. An overview of physiological differences
8 between young and old plants, and the consequences of these differences for O₃ sensitivity, was
9 provided in Section AX9.3.5.3. The discussion below focuses on information relevant to
10 developing exposure-response relationships for mature trees. Information from a few
11 experimental studies, as well as scaling efforts based on physiological characteristics
12 incorporated into models, are discussed in Section AX9.6.

13 Although most studies continue to examine the effects of O₃ on seedlings, during the 1990s
14 some studies examined the effects of O₃ on the response of mature trees. Studies of mature trees
15 demonstrate differences in some aspects of O₃ sensitivity between seedlings and mature trees.
16 For some species, such as red oak, seedlings are less sensitive to O₃ than are mature trees
17 (Hanson et al., 1994; Samuelson and Edwards, 1993; Wullschleger et al., 1996). Both red oak
18 seedlings and genetically related mature trees were exposed to CF, 1×-ambient, or 2×-ambient
19 O₃ exposures in OTCs in Tennessee for two growing seasons (Hanson et al., 1994). Nine large
20 chambers (4.6 × 8.2 m) were used to enclose individual mature trees and standard EPA-style
21 OTCs were used for potted seedlings. Ozone exposures expressed as a 24-h SUM00 were 34,
22 79, and 147 ppm·h in 1992 and 37, 95, 188 ppm·h in 1993 for the sub-ambient, and 2×-ambient
23 treatments. Mature trees had a greater light-saturated net photosynthetic rate and stomatal
24 conductance compared to seedling foliage at physiological maturity. By the end of the growing
25 season, exposure to 1×-ambient and 1×-ambient O₃ reduced the light-saturated net
26 photosynthetic rate and stomatal conductance of mature trees by 25 and 50%, respectively,
27 compared with the CF treatment (35 ppm·h). In seedlings, however, light-saturated net
28 photosynthetic rate and stomatal conductance were less affected by O₃ exposure. The authors
29 concluded that extrapolations of the results of seedling-exposure studies to foliar responses of
30 mature forests without considering differences in foliar anatomy and stomatal response between

1 juvenile and mature foliage may introduce large errors into projections of the O₃ responses of
2 mature trees.

3 In a study of ponderosa pine in California, seedlings and branches of mature trees (in
4 branch chambers) were exposed to O₃ concentrations of 0.5-, 1-, and 2×-ambient O₃
5 concentrations (Momen et al., 1997). Net photosynthetic rate of 1-year-old, but not current-year,
6 foliage was reduced in mature trees but not significantly reduced in seedlings. This effect was
7 not due to alteration of stomatal conductance by O₃. This result contrasts with those with earlier
8 studies of red spruce (Rebbeck et al., 1993).

9 In contrast to the findings for red oak and Ponderosa pine, giant sequoia seedlings had
10 higher rates of stomatal conductance, CO₂-exchange rate, and dark respiration than did mature
11 trees (Grulke and Miller, 1994). As compared to older trees, stomatal conductance was more
12 than 7-fold greater in current-year, and 4-fold greater in 2-year-old, seedlings (Grulke and
13 Miller, 1994). The authors concluded that giant sequoia seedlings are sensitive to atmospheric
14 O₃ until ~5 years of age. Low conductance, high water use efficiency, and compact mesophyll
15 all contribute to a natural O₃ tolerance, or to O₃ defense, or to both, in the foliage of older trees.
16 Similarly, lower stomatal conductance was found in mature Norway spruce in Austria
17 compared to seedlings grown with optimal water and nutrients in a growth chamber (Wieser,
18 1997). In this study, net photosynthetic rate was less sensitive to added O₃ in mature trees
19 compared to seedlings. In a related study, the average rate of O₃ uptake of 17-year-old trees
20 ~0.6 nmol m⁻² s⁻¹, decreasing linearly in older trees, such that rates were only ~0.1 m⁻² s⁻¹ in
21 216-year-old trees (Wieser et al., 1999).

22 Based on a review of studies of stomatal conductance in both seedlings and mature trees,
23 Samuelson and Kelly (2001) concluded that O₃ uptake in oak species, black cherry, sugar maple,
24 and American beech averaged 47% lower in potted seedlings than in mature trees. For evergreen
25 species, they concluded that O₃ uptake in seedlings averaged 26% higher than in mature trees.
26 They also suggested that artifacts introduced by growth in pots confound these differences that
27 exposure-response functions derived from seedlings grown in situ are more applicable to mature
28 trees than are studies of seedlings grown in pots (Samuelson and Kelly, 2001).

29 As discussed above for annual vegetation, it has long been noted that internal O₃ dose is
30 more appropriate than external O₃ exposure for assessing the effects of O₃ on vegetation, because
31 effects occur primarily via the uptake of O₃ through the stomata (Section AX9.2.2). However,

1 external O₃ exposure sometimes has been shown to explain O₃ effects as well or better than
2 calculated internal O₃ dose. For ponderosa pine, Grulke and others (2002) found little difference
3 in the response of net photosynthetic rate and stomatal conductance to O₃ exposure as compared
4 to calculated O₃ uptake; and estimated O₃ uptake by ponderosa pine and O₃ exposure at several
5 sites were highly correlated ($r^2 = 0.92$). For red oak, Hanson and others (Hanson et al., 1994)
6 found that SUM00 explained 83% of the variance in the response of light-saturated
7 photosynthetic rate to O₃ levels, while estimated internal dose explained only 76% of the
8 variance. In this same study, SUM06 explained only 49% of the variance. Due to genetic
9 variation or other factors, individual mature trees will vary in their response to similar O₃
10 exposures. For example, in 125-year-old giant sequoia trees exposed to ~230 ppm·h of O₃ in
11 branch chambers, O₃ uptake in one individual was ~5 mmol m⁻², while in another it was
12 ~9.5 mmol m⁻² (Grulke et al., 1996).

13 Based on these results, stomatal conductance, O₃ uptake, and O₃ effects cannot be assumed
14 to be equivalent in seedlings and mature trees. In general, mature deciduous trees are likely to
15 be more sensitive to O₃ compared to seedlings, while mature evergreen trees are likely to be less
16 sensitive than seedlings. However, even when differences in physiological traits occur,
17 concomitant effects on stem growth may not be detected in the field. Additionally, complex
18 interactions may occur between environmental conditions and O₃ responses; and artifacts may
19 occur for seedling studies, especially for seedlings grown in pots. Finally, competition between
20 species or genotypes within a species can either exacerbate or ameliorate the effects of O₃. Such
21 effects are predicted by models of the growth of mixed species forests, as discussed in Section
22 AX9.6, and various interactions between competitive ability and O₃ effects have been found for
23 aspen clones in the Wisconsin FACE study (McDonald et al., 2002). Section AX9.6 further
24 discusses issues that must be addressed when scaling data from individual mature trees to forests
25 and regions.

27 **AX9.5.6 Studies with the Chemical EDU**

28 The chemical EDU has been used with the goal of protecting plants from O₃ effects
29 without controlling O₃ exposure (Table AX9-20) (U.S. Environmental Protection Agency, 1986,
30 1996). As discussed in Section AX9.1.3.3, the use of EDU has the potential to be a low-cost,

1 practical method of evaluating ambient O₃ exposures on plants grown under natural conditions
2 without the limitations imposed by methodologies such as OTCs (Section AX9.1.3.3). However,
3 because EDU is phytotoxic, and may have effects on plants other than antioxidant protection, it
4 is crucial that the correct dosage for protection from O₃ be determined without the direct effects
5 of EDU. For example, a study in Massachusetts applied EDU to foliage of field-grown bush
6 beans of two lines (S156, R123) and two cultivars (Bush Blue Lake 290, Bush Blue Lake 274)
7 (Elagöz and Manning, 2005a). EDU increased the above-ground biomass of one line but
8 decreased the above-ground biomass in the other line and in one of the cultivars. Other studies
9 have shown that EDU does not always have greater effects at higher O₃ exposures (Ribas and
10 Penuelas, 2000; Tonneijck and Van Dijk, 1997). Such results suggest that it may be difficult to
11 quantify ambient O₃ effects using EDU, because the amount of plant growth or yield expected at
12 a low (background) O₃ concentration cannot be inferred from EDU-treated plants grown at
13 locations with higher O₃ exposures. Unfortunately, although many studies with EDU have been
14 conducted in recent decades, very few have used multiple EDU application levels along with
15 multiple O₃ exposures to characterize the EDU system for a given plant species. Therefore, the
16 text of this section focuses on how data from existing studies can be used for developing or
17 validating exposure-response relationships, rather than reviewing results of all individual studies.
18 Data from individual studies on O₃ exposure, EDU application rates, and the effects of EDU are
19 presented in Table AX9-20. In addition to EDU, sodium erythorbate has been used in a few
20 studies as a protectant chemical. Since very few published studies have used sodium erythorbate
21 and attempts to establish appropriate doses for individual species are even more limited, the use
22 of this chemical is not reviewed here.

23 In summary, it is difficult to use data from existing EDU studies to develop exposure-
24 response relationships or to quantify the effects of ambient O₃ exposure. Despite these
25 limitations, the EDU studies reviewed in previous criteria documents (U.S. Environmental
26 Protection Agency, 1986, 1996) and the more recent studies summarized in Table AX9-20
27 provide another line of evidence that ambient O₃ exposures occurring in many regions of the
28 United States are reducing the growth of crops and trees.

1 **AX9.5.7 Summary**

2 Data published during the last decade support the conclusions of previous criteria
3 documents that there is strong evidence that ambient concentrations of O₃ cause injury and
4 damage to numerous common and economically valuable plant species. For annual vegetation,
5 the data summarized in Table AX9-16 show a range of growth and yield responses both within
6 and among species. Nearly all of these data were derived from studies in OTCs, with only two
7 studies using open-air systems in the UK (Ollerenshaw et al., 1999; Ollerenshaw and Lyons,
8 1999). It is difficult to compare studies that report O₃ exposure using different indices, such as
9 AOT40, SUM06, or 7-h or 12-h mean values. However, when such comparisons can be made,
10 the results of more recent research confirm earlier results summarized in the 1996 O₃ AQCD
11 (U.S. Environmental Protection Agency, 1996). A summary of earlier literature concluded that a
12 7-h, 3-month mean of 49 ppb corresponding to a SUM06 exposure of 26 ppm·h would cause
13 10% loss in 50% of 49 experimental cases (Tingey et al., 1991). More recent data summarized
14 in Table AX9-16 support this conclusion, and more generally indicate that ambient O₃ exposures
15 can reduce the growth and yield of annual species. Some annual species such as soybean are
16 more sensitive, and greater losses may be expected (Table AX9-16). Thus, the more recent
17 scientific literature supports the conclusions of the 1996 O₃ AQCD (U.S. Environmental
18 Protection Agency, 1996) that ambient O₃ concentrations are probably reducing the yield of
19 major crops in the United States.

20 Much research in Europe has used the AOT40 exposure statistic, and substantial effort has
21 gone into developing Level-1 critical levels for vegetation using this index. Based on regression
22 analysis of 15 OTC studies of spring wheat, including one study from the United States and
23 14 from locations ranging from southern Sweden to Switzerland, an AOT40 value of 5.7 ppm·h
24 was found to correspond to a 10% yield loss, and a value of 2.8 ppm·h corresponded to a 5%
25 yield loss (Fuhrer et al., 1997). Because a 4 to 5% decrease could be detected with a 99%
26 confidence level, a critical level of an AOT40 value of 3 ppm·h was selected in 1996
27 (Kärenlampi and Skärby, 1996).

28 In addition to likely reductions in crop yield, O₃ may also reduce the quality or nutritive
29 value of annual species. Many studies have shown effects of O₃ on various measures of plant
30 organs that affect quality, with most studies focusing on characteristics important for food or
31 fodder. These studies indicate that there may be economically important effects of ambient O₃

1 on the quality of crop and forage species. Previous criteria documents have concluded that
2 visible symptoms on marketable portions of crops and ornamental plants can occur with seasonal
3 7-h mean O₃ exposures of 40 to 100 ppb (U.S. Environmental Protection Agency, 1978, 1986,
4 1996). The more recent scientific literature does not refute this conclusion.

5 The use of OTCs may reverse the usual vertical gradient in O₃ that occurs within a few
6 meters above the ground surface (Section AX9.1). This reversal suggests that OTC studies may
7 somewhat overestimate the effects of an O₃ concentration measured several meters above the
8 ground. However, such considerations do not invalidate the conclusion of the 1996 AQCD (U.S.
9 Environmental Protection Agency, 1996) that ambient O₃ exposures (Tables AX9-13 and
10 AX9-21) are sufficient to reduce the yield of major crops in the United States.

11 As for single-season agricultural crops, yields of multiple-year forage crops are reduced
12 at O₃ exposures that occur over large areas of the United States. This result is similar to that
13 reported in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996). When species
14 are grown in mixtures, O₃ exposure can increase the growth of O₃-tolerant species and
15 exacerbate the growth decrease of O₃-sensitive species (e.g., Ashmore and Ainsworth, 1995;
16 Fuhrer et al., 1994). Because of this competitive interaction, the total growth of the mixed-
17 species community may not be affected by O₃ exposure (Ashmore and Ainsworth, 1995; Barbo
18 et al., 1998; Fuhrer et al., 1994). However, in some cases, mixtures of grasses and clover species
19 have shown significant decreases in total biomass growth in response to O₃ exposure in studies
20 in the United States (Heagle et al., 1989; Kohut et al., 1988) and in Sweden (Pleijel et al., 1996).
21 In Europe, a provisional critical level for herbaceous perennials of an AOT40 value of 7 ppm-h
22 over 6 months has been proposed to protect sensitive plant species from adverse effects of O₃.

23 For deciduous tree species, recent evidence from free air exposure systems and OTCs
24 supports results observed previously in OTC studies. For example, a series of FACE studies
25 undertaken in Rhinelander, WI (Isebrands et al., 2000, 2001) showed that O₃-symptom
26 expression was generally similar in OTCs, FACE, and also at sites along an ambient O₃ gradient,
27 supporting the previously observed variation among aspen clones using OTCs (Karnosky et al.,
28 1999). This study also found no effects on sugar maple growth after 3 years, but in years 4 to 7
29 found significant growth reduction due to O₃ (Karnosky et al., 2005). These results are important
30 because, they indicate results obtained from OTCs are supported by results from free air
31 exposure systems and also that more than 3 years may be required to adequately investigate

1 effects of O₃ on the growth of tree species. As has been observed in previous criteria documents,
2 root growth often is found to be the most sensitive biomass response indicator to O₃.

3 Results reported since 1996 support the conclusion of the 1996 O₃ AQCD (U.S.
4 Environmental Protection Agency, 1996) that deciduous trees are generally less sensitive to O₃
5 than are most annual plants, with the exception of a few very sensitive genera such as *Populus*
6 and sensitive species such as black cherry. However, the data presented in Table AX9-18
7 suggest that ambient O₃ exposures that occur in the United States can potentially reduce the
8 growth of seedlings of deciduous species. Results from multiple-year studies sometimes show a
9 pattern of increased effects in subsequent years. In some cases, however, growth decreases due
10 to O₃ may become less significant or even disappear over time. While some mature trees show
11 greater O₃ sensitivity in physiological parameters such as net photosynthetic rate than do
12 seedlings, these effects may not translate into measurable reductions in biomass growth.
13 However, because even multiple-year experiments do not expose trees to O₃ for more than a
14 small fraction of their life span, and because competition may in some cases exacerbate the
15 effects of O₃ on individual species, determining effects on mature trees remains a significant
16 challenge.

17 In Europe, a Level I critical level has been set for forest trees based on OTC studies of
18 European beech seedlings. A critical level was defined as an AOT40 value of 10 ppm·h for
19 daylight hours for a 6-month growing season (Kärenlampi and Skärby, 1996). However, other
20 studies show that some species such as silver birch may be more sensitive to O₃ compared to
21 beech (Pääkkönen et al., 1996).

22 For evergreen tree species, as for other tree species, the O₃ sensitivity of different
23 genotypes and different species varies widely. Based on studies with seedlings in OTCs, major
24 species in the United States are generally less sensitive than are most deciduous trees, and
25 slower- growing species are less sensitive than are faster-growing species. Interacting stresses
26 such as competition stress may increase the sensitivity of trees to O₃. As for deciduous species,
27 most experiments with evergreen species have only covered a small portion of the life span of a
28 tree and have been conducted with seedlings, making estimating effects on mature trees difficult.

29 For all types of perennial vegetation, cumulative effects over more than one growing
30 season may be important; furthermore, studies for only a single season may underestimate
31 effects. Mature trees may be more or less sensitive to O₃ than are seedlings, depending on the

1 species, but information on physiological traits may be used to predict some such differences. In
2 some cases, mature trees may be more sensitive to O₃ than seedlings due to differences in their
3 gas exchange rates, growth rates, greater cumulative exposures, or due to the interaction of O₃
4 stress with other stresses.

5

6

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54

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

2 **AX9.6.1 Introduction**

3 The preceding section on species-level responses (AX9.5) provides a lead-in to address the
4 response of ecosystems to O₃. The conclusion of the 1996 O₃ AQCD (U.S. Environmental
5 Protection Agency, 1996) was that aside from the results from the San Bernardino NF, there was
6 no direct evidence that O₃ is altering natural ecosystems in the United States. This conclusion is
7 generally valid today, except that our understanding of the effects of O₃ in the San Bernardino
8 NF has been tempered by additional understanding of the complicating role that N deposition
9 plays in this system. Despite the lack of any new, direct information linking O₃ with ecosystem
10 changes, numerous publications since 1996 have highlighted ways in which O₃ may affect
11 ecosystem structure and/or function. This section addresses new and (where appropriate) older
12 literature in order to illustrate possible shifts in energy or material flow through ecosystems as a
13 result of O₃ exposure.

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

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

29 Ecosystems are functionally highly integrated. Changes in one part of an ecosystem, such
30 as the primary producer component, may have consequences for connected parts, such as the
31 consumer and decomposer components. For example, when needles are shed prematurely as a

1 result of O₃ exposure, successional development of phyllosphere fungi inhabiting the surface of
2 Ponderosa pine needles may be truncated (Bruhn, 1980). In addition, decomposer populations in
3 the litter layer may be capable of higher rates of decomposition, due to the higher N content of
4 the younger age classes of needles falling from O₃-damaged pines (Fenn and Dunn, 1989).
5 Because ecological systems integrate the effects of many influences, the results of O₃ exposure
6 may depend on co-occurring influences that predispose an ecosystem to stress (Colls and
7 Unsworth, 1992). One important change in our thinking since the 1996 O₃ AQCD is that, at the
8 high levels of O₃ exposure that are known to result in detectable plant responses (>250 ppm h
9 accumulated over a growing season), N deposition must also be considered as a concurrent
10 stressor. Both O₃ exposure and increased N deposition can cause changes in N cycling and
11 compartmentalization within ecosystems.

12 The vast majority of O₃-effects literature addresses individual species responses (see
13 Section AX9.6.4.3), as was also true in 1996 (U.S. Environmental Protection Agency, 1996).
14 This section differs from the preceding one in that the physiological stress of individual species
15 is considered only within the context of its natural ecosystem. Changes in function at the
16 individual level propagate through the higher levels of organization, resulting in changes in
17 ecosystem structure and function. However, since ecosystem-level responses result from the
18 interaction of organisms with one another and with their physical environment, it takes longer
19 for a change to develop to a level of prominence at which it can be identified and measured. The
20 paucity of scientific literature on O₃ effects at the ecosystem level is a result of both the
21 complexity of ecological systems, and long response times. In addition, “indirect” effects of O₃
22 on plants (e.g., effects that alter the plants’ ability to integrate environmental stresses) may be
23 more important than the direct effects on photosynthesis and respiration at the leaf level
24 (Johnson and Taylor, 1989).

25 A conceptual framework (see Table AX9-22) for discussing the effects of O₃ on
26 ecosystems was developed by the EPA Science Advisory Board (Young and Sanzone, 2002).
27 Their six essential ecological attributes (EEAs) include landscape condition, biotic condition,
28 organism condition, ecological processes, hydrological and geomorphological processes, and
29 natural disturbance regimes (see Table AX9-22). The major ecological effects of O₃, and gaps in
30 our knowledge of O₃ exposure effects at the ecosystem level, are summarized at the end of this
31 chapter. While the main focus is O₃ effects newly described since the 1996 O₃ AQCD (U.S.

Table AX9-22. Essential Ecological Attributes for Natural Ecosystems Affected by O₃

Category	Species	Condition Measures	References
Landscape Condition			
• Habitat Types			
Biotic Condition			
• Ecosystems and Communities	Mixed conifer forest	Community composition, Stand structure	Miller et al. (1989) Miller and McBride (1999a)
Community Extent and Composition	<i>Pinus ponderosa</i>	Relative abundance	Miller (1973); Arbaugh et al. (2003)
	Grassland communities	Species composition	Ashmore et al. (1995); Ashmore and Ainsworth (1995)
	Coastal sage scrub	Species cover, richness, equitability	Westman (1979, 1981)
	Early successional plant community	Species richness, diversity, evenness	Barbo et al. (1998)
	<i>Populus tremuloides</i> and <i>Betula papyrifera</i>	Soil microbial community	Phillips et al. (2002)
	<i>Pinus ponderosa</i>	Soil microbial community	Scagel and Andersen (1997)
	<i>Pinus taeda</i>	Fungal morphotypes	Edwards and Kelly (1992); Qui et al. (1993)
Trophic Interactions			
<i>Insects</i>	<i>Pinus ponderosa</i>	Bark beetle severity	Cobb et al. (1968)
	<i>Pinus ponderosa</i>	Bark beetle productivity and predator/parasitoid density	Dahlsten et al. (1997)
	<i>Populus tremuloides</i>	Blotch leaf miner performance	Kopper and Lindroth (2003a)
	<i>Populus tremuloides</i>	Aphid/natural enemy abundance	Percy et al. (2002)
	<i>Populus tremuloides</i>	Forest tent caterpillar/paratisoid performance	Percy et al. (2002); Holton et al. (2003)
<i>Diseases</i>	<i>Populus hybrids</i>	<i>Septoria</i> occurrence	Woodbury et al. (1994)
	<i>Populus hybrids</i>	Rust occurrence	Beare et al. (1999a)
	<i>Populus tremuloides</i>	Rust occurrence	Karnosky et al. (2002)
	<i>Picea abies</i> and <i>Picea sitchensis</i>	Needle fungi	Magan et al. (1995)
	<i>Pinus ponderosa</i>	Root disease × O ₃ interactions	Fenn et al. (1990)
	<i>Pinus taeda</i>	Canker dimensions	Carey and Kelley (1994)
	<i>Pinus sylvestris</i> / mycorrhizae	Disease susceptibility	Bonello et al. (1993)

**Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems
Affected by O₃**

Category	Species	Condition Measures	References
<i>Community Dynamics</i>	<i>Pinus ponderosa/Abies concolor/Calocedrus decurrens</i>	Abundance	Minnich et al. (1995)
	<i>Populus tremuloides</i>	Competitive status	McDonald et al. (2002)
	<i>Pinus ponderosa/Elymus glaucus</i>	O ₃ sensitivity	Andersen et al. (2001)
	<i>Pinus taeda</i> /diverse community	Tree growth	Barbo et al. (2002)
• Species and Populations			
<i>Population Size</i>	<i>Pinus strobus</i>	Mortality	Karnosky (1981)
	<i>Pinus ponderosa</i>	Mortality	Carroll et al. (2003)
<i>Genetic Diversity/ Population Structure</i>	<i>Lupinus bicolor</i>	% population sensitive	Dunn (1959)
	<i>Populus tremuloides</i>	% population sensitive	Berrang et al. (1986, 1989, 1991)
	<i>Trifolium repens</i>	% population sensitive	Heagle et al. (1991)
	<i>Plantago major</i>	% population sensitive	Davison and Reiling (1995); Reiling and Davison (1992b); Lyons et al. (1997)
<i>Population Dynamics</i>	<i>Trifolium repens</i>	Adaptation	Heagle et al. (1991)
	<i>Plantago major</i>	Population changes over time	Davison and Reiling (1995)
<i>Organism Condition</i>			
• Visible Symptoms			
	<i>Pinus ponderosa</i>	Foliar symptoms	Grulke and Lee (1997); Arbaugh et al. (1998); Salardino and Carroll (1998); Temple et al. (1992) Grulke et al. (2003b)
	<i>Pinus jeffreyi</i>	Foliar symptoms	Patterson and Rundel (1995); Salardino and Carroll (1998); Fredericksen et al. (1995, 1996); Chappelka et al. (1997, 1999a,b)
	<i>Prunus serotina</i>	Foliar symptoms	Hildebrand et al. (1996); Ghosh et al. (1998); Lee et al. (1999); Ferdinand et al. (2000); Schaub et al. (2003)
	<i>Liriodendron tulipifera</i>	Foliar symptoms	Yuska et al. (2003); Somers et al. (1998); Hildebrand et al. (1996)
	<i>Sassafras albidum</i>	Foliar symptoms	Chappelka et al. (1999a,b)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

Category	Species	Condition Measures	References
<i>Organism Condition (cont'd)</i>			
• Visible Symptoms (cont'd)	<i>Populus nigra</i> , <i>Fraxinus excelsior</i> and <i>Prunus avium</i>	Foliar symptoms	Chappelka et al. (1999a); Novak et al. (2003)
	<i>Fagus sylvatica</i>	Foliar symptoms	Gerosa et al. (2003); Vollenweider et al. (2003b)
	<i>Fraxinus americana</i>	Foliar symptoms	Schaub et al. (2003)
	Grassland species	Foliar symptoms	Bungener et al. (1999a)
	Herbaceous species	Foliar symptoms	Bergmann et al. (1999)
	<i>Asclepias exaltata</i>	Foliar symptoms	Chappelka et al. (1997)
	<i>Rudbeckia laciniata</i> and <i>Verbesina occidentalis</i>	Foliar symptoms	Chappelka et al. (2003)
• Physiological Status	<i>Asclepias incarnata</i>	Foliar symptoms	Orendovici et al. (2003)
	<i>Pinus halepensis</i>	Allometry	Wellburn and Wellburn (1994)
	<i>Populus tremuloides</i>	Crown architecture	Dickson et al. (2001)
	<i>Betula pendula</i>	Crown architecture	Kull et al. (2003)
	<i>Fagus sylvatica</i>	Crown architecture	Stribley and Ashmore (2002)
	<i>Populus tremuloides</i>	Root dry weight	Coleman et al. (1996)
	<i>Pinus ponderosa</i>	Root/shoot ratio	Grulke et al. (1998a); Grulke and Balduman (1999)
	<i>Festiva ovina</i>	Root/shoot ratio	Warwick and Taylor (1995)
	<i>Betula pubescens</i>	Root/shoot ratio	Mortensen (1998)
	<i>Populus tremuloides</i> × <i>Populus tremula</i>	Root/shoot ratio	Landolt et al. (2000); Paludan-Müller et al. (1999)
	<i>Populus tremuloides</i>	Leaf area index	Oksanen et al. (2001)
	<i>Pinus ponderosa</i>	Carbon allocation to mycorrhizae	Neufeld et al. (1995); Wiltshire et al. (1996); Andersen and Rygielwicz (1995a,b)
	<i>Betula pendula</i>	Decreased winter bud formation	Karnosky et al. (2003a)
	<i>Betula pendula</i>	Delayed bud break	Oksanen (2003a,b)
<i>Acer saccharum</i>	Early bud break	Prozherina et al. (2003); Bertrand et al. (1999)	

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

Category	Species	Condition Measures	References
• Reproductive Status	<i>Apocynun androsaemifolium</i>	Flowering time	Bergweiler and Manning (1999)
	<i>Buddleia davidii</i>	Flowering time	Findley et al. (1997)
	<i>Rubus cuneifolius</i>	Pollen germination	Chappelka (2002)
	<i>Plantago major</i>	Pollen tube elongation	Stewart (1998)
	<i>Fragaria</i> × <i>ananassa</i>	Fruit yield	Drogoudi and Ashmore (2000, 2001)
	<i>Plantago major</i>	Seed yield	Lyons and Barnes (1998); Pearson et al. (1996); Reiling and Davison (1992a); Whitfield et al. (1997)
	Understory herbs	Seed yield	Harward and Treshow (1975)
<i>Ecological Processes</i>			
• Energy Flow			
Primary Production	<i>Pinus ponderosa</i>	Photosynthesis	Miller et al. (1969); Clark et al. (1995); Takemoto et al. (1997); Grulke et al. (2002b)
	<i>Pinus ponderosa</i>	Needle retention	Temple et al. (1993)
	<i>Populus tremuloides</i>	Photosynthesis	Coleman et al. (1995ab); Noormets et al. (2001a,b); Sharma et al. (2003); Karnosky et al. (2003a); Oksanen (2003a,b)
	<i>Betula pendula</i>	Photosynthesis/conductance	Matyssek et al. (2002)
	<i>Betula pendula</i>	Stem respiration and radial growth	Kelting et al. (1995)
	<i>Quercus rubra</i>	Root turnover	Coleman et al. (1996)
	<i>Populus tremuloides</i>	Soil respiration	King et al. (2001); Andersen and Scagel (1997); Coleman et al. (1995a)
	<i>Pinus ponderosa</i>	Soil respiration	Scagel and Andersen (1997); Andersen (2000); Samuelson and Kelly (1996)
	<i>Quercus rubra</i>	Carbon partitioning and allocation	Andersen et al. (1997); Grulke et al. (1998a)
	<i>Populus tre muloides</i>	Carbon allocation	Grulke and Balduman (1999)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

Category	Species	Condition Measures	References
Primary Production (cont'd)	<i>Pinus ponderosa</i>	Carbon allocation	Grulke et al. (2001)
	<i>Betula pendula</i>	Carbon allocation	Karlsson et al. (2003); Oksanen and Saleem (1999); Saleem et al. (2001)
	<i>Fragaria vesca</i>	Carbon allocation	Manninen et al. (2003)
	<i>Pinus taeda</i>	Root respiration	Edwards (1991)
	<i>Lespedeza cuneata</i> and <i>Schizacbyrium scoparium</i>	Yield	Powell et al. (2003)
	<i>Liriodendron tulipifera</i>	Radial growth	Somers et al. (1998); Vollenweider et al. (2003a)
	<i>Prunus serotina</i>	Radial growth	Somers et al. (1998)
	<i>Pinus jeffreyi</i>	Radial growth	Peterson et al. (1987)
	<i>Pinus ponderosa</i>	Radial growth (no effect)	Peterson et al. (1993)
	<i>Pinus strobus</i>	Radial growth	Bartholomay et al. (1997)
	<i>Pinus taeda</i>	Radial growth	McLaughlin and Downing (1995; 1996)
	<i>Fagus sylvatica</i>	Stem volume	Braun et al. (1999)
	<i>Picea abies</i>	Stem volume	Wallin et al. (2002)
	<i>Populus tremuloides</i>	Volume growth	Isebrands et al. (2001)
	Net Ecosystem Production	Northern hardwoods	NPP estimates
Northern hardwoods		Biomass estimates	Hogsett et al. (1997)
<i>Growth Efficiency</i>	<i>Plantago major</i>	Relative growth rate	Davison and Reiling (1995); Lyons et al. (1997); Reiling and Davison (1992b); Davison and Barnes (1998)
	Grassland species	Relative growth rate	Bungener et al. (1999b)
	Native herbs	Relative growth rate	Warwick and Taylor (1995)
	Grasses and herbs	Relative growth rate	Pleijel and Danielsson (1997)
	<i>Populus tremuloides</i>	Relative growth rate	Yun and Laurence (1999)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

Category	Species	Condition Measures	References
Growth Efficiency (Cont'd)	<i>Fagus sylvatica</i>	Relative growth rate	Bortier et al. (2000a)
	<i>Picea abies</i>	Relative growth rate	Karlsson et al. (2002)
	<i>Prunus serotina</i>	Relative growth rate	Lee et al. (2002)
• Material Flow <i>Organic Carbon Cycling</i>	<i>Populus tremuloides</i> and <i>Betula papyrifera</i>	Altered foliar C:N ratio and N resorption efficiency	Lindroth et al. (2001)
	<i>Andropogon virginicus</i> and <i>Rubus cuneifolius</i>	Litter decomposition rate	Kim et al. (1998)
	<i>Liriodendron tulipera</i>	Litter decomposition rate	Scherzer et al. (1998)
	<i>Populus deltoides</i>	Litter decomposition rate	Findlay and Jones (1990)
	<i>Pinus ponderosa</i>	Litter decomposition rate	Fenn and Dunn (1989)
	<i>Pinus sylvestris</i>	Litter decomposition (no effect)	Kainulainen et al. (2003)
	<i>Nitrogen Cycling</i>	<i>Pinus ponderosa</i>	Altered foliar N
<i>Pinus ponderosa</i>		Foliar N and O ₃ exposure no effects	Bytnerowicz et al. (1990)
<i>Pinus taeda</i>		Altered foliar N metabolism	Manderscheid et al. (1992)
<i>Prunus serotina</i> and <i>Liriodendron tulipifera</i>		Altered foliar N	Boerner and Rebbeck (1995)
<i>Other Nutrient Cycling</i>	<i>Picea sitchensis</i> and <i>Pinus sylvestris</i>	Foliar leaching no effect	Skeffington and Sutherland (1995)
	<i>Pinus ponderosa</i>	Nutrient availability and O ₃	Bytnerowicz et al. (1990)
<i>Hydrology and Geomorphology</i>			
• Water Budget	<i>Picea rubens</i>	Water-use efficiency no effect	Laurence et al. (1997)
	<i>Pinus armandi</i>	Water-use efficiency	Shan et al. (1996)
	<i>Pinus jeffreyi</i>	Canopy transpiration	Grulke et al. (2003a)
	<i>Picea abies</i>	Transpiration (xylem sap flow)	Maier-Maercker (1997)
	<i>Fraxinus excelsior</i>	Water stem flow	Wiltshire et al. (1994)
	<i>Betula pendula</i>	Water-use efficiency	Maurer and Matyssek (1997)
	<i>Populus</i> hybrids	Water-use efficiency	Reich and Lassoie (1984)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

Category	Species	Condition Measures	References
<i>Natural Disturbance Regimes</i>			
• Frequency	<i>Pinus ponderosa</i>	Frequency of fire	McBride and Laven (1976); Minnich et al. (1995); Miller and McBride (1999)
	<i>Pinus ponderosa</i>	Occurrence of bark beetle outbreaks	Pronos et al. (1999); Dahlsten et al. (1997)
• Intensity	<i>Picea sitchensis</i>	Winter damage	Lucas et al. (1988)
	<i>Pinus halepensis</i>	Reduced winter damage	Wellburn and Wellburn (1994)
	<i>Picea rubens</i>	Freezing tolerance	Waite et al. (1994)
	<i>Fagus sylvatica</i>	Drought stress	Pearson and Mansfield (1993, 1994)
	<i>Picea abies</i>	Drought stress	Maier-Maercker (1998); Maier-Maercker and Koch (1992)
• Extent	<i>Pinus ponderosa</i>	Fire intensity	Miller and McBride (1999a)
	<i>Pinus ponderosa</i>	Extent of bark beetle attack	Minnich et al. (1995)
• Duration	<i>Pinus ponderosa</i>	Duration of bark beetle attack	Minnich et al. (1995)

1 Environmental Protection Agency, 1996), many key historical papers are cited to demonstrate
 2 ecosystem response, particularly where they remain the only examples in the literature.
 3 Although the vast majority of published studies focus on individuals, six case studies (five
 4 field examples and one FACE experiment) have measured several ecosystem components
 5 simultaneously to better understand the overall ecosystem response to O₃. We provide an
 6 overview of these six studies up-front to provide a context for the subsequent discussion on
 7 possible ecosystem effects on an EEA basis.

8
 9 **AX9.6.2 Case Studies**

10 **AX9.6.2.1 Valley of Mexico**

11 The first evidence of air pollution impacts on vegetation in the Valley of Mexico (Mexico
 12 City Air Basin) were observations of foliar injury symptoms in bioindicator plants attributed
 13 to O₃, PAN, SO₂, and possibly other pollutants (De Bauer, 1972). Subsequently, O₃ injury to

1 foliage and crowns of pine trees were reported in forests to the south and southwest of Mexico
2 City (De Bauer and Hernández-Tejeda, 1986; De Bauer and Krupa, 1990). Ozone is considered
3 to be the pollutant with the most severe impacts on vegetation within the Mexico City urban
4 zone and in forests downwind of the city. *Pinus hartwegii* is the most O₃-sensitive pine species
5 and is severely impacted by high O₃ exposures encountered to the south/southwest of the Mexico
6 City metropolitan area (Miller et al., 1994). The potential for O₃ injury is particularly high in
7 this area, because O₃ levels are high during the summer rainy season when soil moisture
8 availability and stomatal conductance are greatest; these factors enhance O₃ uptake and injury.
9 Decline of *Abies religiosa* (oyamel) in the Desierto de los Leones NP is a well-known example
10 of dramatic dieback and mortality of entire forest stands due primarily to air pollution stress
11 (Alvarado et al., 1993). Other factors, such as a lack of stand thinning, also contribute to forest
12 decline. Lead in automobile gasoline was phased out in 1990, and foliar concentrations of heavy
13 metals in forest species are not now at phytotoxic levels (Fenn and De Bauer, 1999). Sulfur
14 dioxide concentrations decreased in the early 1990s as a result of regulatory mandates limiting
15 their emissions. Sensitive plants in the northeast and northwest sectors of the Mexico City urban
16 zone where concentrations are highest may still be impacted by exposure to ambient SO₂ levels.
17 Deposition of ionic forms of N and S are high in forested areas southwest of Mexico City.
18 However, the effects of these chronic nutrient inputs to the forest are only beginning to be
19 investigated and understood.

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

28 29 **AX9.6.2.2 San Bernardino Mountains**

30 The San Bernardino Mountains lie east of the Los Angeles Air Basin in California, and
31 significant levels of pollution have been transported into the mountain range, including into a

1 Class I wilderness area. The effects of O₃ exposure on the mixed conifer forest of the
2 San Bernardino Mountains is perhaps the longest and most thoroughly documented O₃
3 ecological effects evaluation (Miller and McBride, 1999a). In this classic case study linking
4 tropospheric O₃ exposure to damage to an entire forest ecosystem (U.S. Environmental
5 Protection Agency, 1996) (Table AX9-23), Miller et al. (1963) first identified the unique foliar
6 chlorotic mottle that was occurring on two of the dominant tree species, *Pinus ponderosa* and
7 *P. jeffreyi*. Levels of O₃ averaging 100 to 120 ppb over 24 h with 1-h peaks well into the 200 ppb
8 range were common in the region in the 1960s and 1970s (Miller and McBride, 1999a). Single-
9 hour peak values have declined in recent years due to heavily regulated pollution control
10 (Arbaugh et al., 1998; Lee et al., 2003; Takemoto et al., 2001).

11 Since the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) was written, the
12 concurrent role of N deposition in modifying ecosystem response to O₃ exposure in the San
13 Bernardino Mountains has been further elucidated (Arbaugh et al., 2003; Bytnerowicz et al.,
14 1999; Bytnerowicz, 2002; Fenn et al., 1996; Takemoto et al., 2001). Both O₃ exposure and N
15 deposition reduce foliar retention (Grulke and Balduman, 1999) and alter tissue chemistry of
16 both needles and litter (Poth and Fenn, 1998). In addition, confounding factors such as drought
17 and fire suppression add to the complexity of ecosystem response (Arbaugh et al., 2003;
18 Minnich et al., 1995; Takemoto et al., 2001). Extensive crown injury measurements have also
19 been made, linking ambient O₃ exposure data to chlorotic mottle and fascicle retention (Arbaugh
20 et al., 1998). Ozone exposure and N deposition reduce carbon allocation to stems and roots
21 (Grulke et al., 1998a, 2001), further predisposing trees to drought stress, windthrow, root
22 diseases, and insect infestation (Takemoto et al., 2001). Increased mortality of susceptible tree
23 species (Ponderosa and Jeffrey pine) has shifted community composition toward white fir and
24 incense-cedar (*Abies concolor*, *Calocedrus decurrens*) and has altered forest stand structure
25 (Miller et al., 1989) (Table AX9-25). Ozone exposure is implicated in projected changes in
26 stand composition (McBride and Laven, 1999) toward a predominance of oaks, rather than
27 mixed conifer forests. Forest understory species have also been affected (Temple, 1999). These
28 individual species responses collectively have affected trophic structure and food web dynamics
29 (Dahlsten et al., 1997; Pronos et al., 1999), as well as C and N cycling (Arbaugh et al., 2003)
30 (Table AX9-24).

Table AX9-23. Case Studies Demonstrating the Ecological Effects of O₃

Study	Keystone Species	Study Type	Period Studied	Key Ecological Findings
Valley of Mexico	<i>Pinus hartwegii</i> , <i>Abies religiosa</i>	Field transects	35 yrs	<ul style="list-style-type: none"> • 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	<i>Pinus ponderosa</i> , <i>P. jeffreyi</i>	Field transects	40 yrs	<ul style="list-style-type: none"> • Community composition changes (Arbaugh et al., 2003; Miller, 1973; Minnich et al., 1995) • Population changes (McBride and Laven, 1999) • O₃-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 O₃, drought, N deposition (Fenn et al., 1996; Grulke, 1999; Takemoto et al., 2001) • Altered carbon cycling (Arbaugh et al., 1999)
Sierra Nevada Mountains	<i>Pinus ponderosa</i> , <i>P. jeffreyi</i>	Field	35 yrs	<ul style="list-style-type: none"> • 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 O₃ flux (Bauer et al., 2000; Goldstein et al., 2003; Panek et al., 2002) • Cumulative O₃ effects (Takemoto et al., 1997) • Canopy level responses (Grulke et al., 2003a,b) • Population changes (Carroll et al., 2003)
Appalachian Mountains	<i>Fraxinus americana</i> , <i>Liriodendron tulipifera</i> , <i>Pinus strobus</i> , <i>Prunus serotina</i>	Field	25 yrs	<ul style="list-style-type: none"> • 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

Study	Keystone Species	Study Type	Period Studied	Key Ecological Findings
Aspen FACE	<i>Acer saccharum</i> , <i>Betula papyrifera</i> , <i>Populus tremuloides</i>	Open-air O ₃ exposure	6 yrs	<ul style="list-style-type: none"> • Competitive interactions (McDonald et al., 2002) • O₃-aspen-rust interaction (Karnosky et al., 2002) • Plant-insect interactions (Holton et al., 2003; Percy et al., 2002) • C and N cycling (King et al., 2001; Lindroth et al., 2001) • Moderation of CO₂ responses by O₃ (Isebrands et al., 2001; Karnosky et al., 2003b; McDonald et al., 2002; Wustman et al., 2001)
Plantago	<i>Plantago major</i>	Field	20 yrs	<ul style="list-style-type: none"> • Population structure (Davison and Reiling, 1995; Lyons et al., 1997) • O₃ resistance (Reiling and Davison, 1992c) • Adaptation (Davison and Reiling, 1995)
Carpathian Mountains	<i>Pinus sylvestris</i> , <i>Picea abies</i>	Field	15 yrs	<ul style="list-style-type: none"> • Significant foliar injury • Community composition changes • Species diversity changes

1

2 **AX9.6.2.3 Sierra Nevada Mountains**

3 Like the San Bernardino Mountains, the western slope of the Sierra Nevada Mountains in
 4 central and southern California has also been exposed to elevated O₃ for a long time, although
 5 the effects have been much less.. Symptoms of O₃ injury have been found on Ponderosa and
 6 Jeffrey pines in all of the Sierra Nevada national forests and parks (Carroll et al., 2003). First
 7 identified as a problem in the 1970s (Miller et al., 1972), elevated O₃ with daytime means of
 8 60-80 ppb are common (Bauer et al., 2000; Böhm et al., 1995; Bytnerowicz et al., 2002c; Panek
 9 et al., 2002). The west-slope Sierra Nevada forests are also exposed to a wide range of
 10 additional gaseous and particulate pollutants, including various S and N compounds
 11 (Bytnerowicz et al., 1999; Fenn et al., 2003b; Takemoto et al., 2001), but at levels much lower
 12 than in the San Bernardino Mountains. Typical O₃-induced visible foliar symptoms, including
 13 chlorotic mottle, chlorophyll degradation, and premature senescence, are commonly found

Table AX9-24. The Most Comprehensively Studied Effects of O₃ on Natural Ecosystem are for the San Bernardino Mountain Forest Ecosystem. Citations Focus on Research Published Since U.S. EPA (U.S. Environmental Protection Agency, 1996).

Pollutant Occurrence	Reference
O ₃ exposure and N deposition	Fenn et al. (1996; 2000); Grulke et al. (1998a, 2003a); Bytnerowicz et al. (1999)
Cellular, Biochemical	
Foliar pigments Antioxidants	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 O ₃ flux Foliar nutrients	Grulke (1999); Grulke et al. (2002a,b); Grulke and Retzlaff (2001)
Whole Organism	
Growth/Biomass <ul style="list-style-type: none"> • Above-ground • Below-ground • Root/shoot ratio • Carbon allocation • Crown vigor 	Grulke and Balduman (1999) Grulke et al. (1998a); Grulke and Balduman (1999); Grulke and Balduman (1999); Grulke et al. (2001); Arbaugh et al. (1998); 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 <ul style="list-style-type: none"> • Bark beetle/predators • Disease occurrence • Litter decomposition 	Dahlsten et al. (1997); Pronos et al. (1999) Miller and Rechel (1999); Pronos et al. (1999)
Disturbance	
<ul style="list-style-type: none"> • Bark beetle occurrence • Fire frequency 	Miller and McBride (1999b); Minnich et al. (1995); Minnich (1999)

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).

	O ₃	O ₃ + N deposition	O ₃ + Drought	References
<i>Foliar Biochemistry and Tissue Chemistry</i>				
Total ascorbate	d ¹	d	i	Grulke et al. (2003b); Tausz et al. (2001)
Dehydroascorbate	i	n.d.	d	Grulke et al. (2003b)
Total glutathione	d	i	d	Tausz et al. (2001)
Oxidized glutathione	i	i	d	Tausz et al. (2001)
α Carotenoids	i	n.d.	d	Grulke et al. (2003b)
Foliar nitrogen	d	i	d	Grulke et al. (1998a); Grulke and Lee (1997); Poth and Fenn (1998)
C:N ratio of foliage ²	i	n.d.	d	Poth and Fenn (1998) Grulke et al. (2003b)
Starch	n.d.	d	i	Grulke et al. (2001)
Chlorophyll content	d	id	d	Grulke et al. (1998b, 2003b); Grulke and Lee (1997); Takemoto et al. (1997)(Grulke (1999); Tausz et al. (2001)
<i>Gas Exchange</i>				
A _{max} lower canopy	n.d.	i	d	Grulke (1999); Grulke et al. (2002b); Grulke and Retzlaff (2001); Panek (2004)
A _{max} whole canopy	d	n.d.	d	Grulke et al. (2003b); Panek and Goldstein (2001)
A _{max} seedlings	di	n.d.	n.d.	Grulke and Retzlaff (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)
O ₃ flux	d	n.s.	d	Panek et al. (2002, 2003); Panek and Goldstein (2001) Grulke et al. (2002a, 2004)

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).

	O ₃	O ₃ + N deposition	O ₃ + Drought	References
<i>Growth and Productivity</i>				
Foliar biomass	n.d.	i	d	Grulke and Balduman (1999)
Height growth	n.d.	i	d	Grulke and Balduman (1999)
Bole diameter growth	d	i	d	Grulke and Balduman (1999)
Fine root biomass	d	d	i	Grulke et al. (1998a)
Leaf Surfaces				
Stomatal occlusion	i	n.d.	n.d.	Bytnerowicz et al. (1999); Bytnerowicz and Turunen (1994)
Trophic Interactions				
Bark beetle	n.s.	i	i	Pronos et al. (1999)
<i>Ecosystem Level</i>				
Competitive indices	n.d.	d	i	Miller and Rechel (1999)

¹Responses 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).

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

1 on O₃-sensitive genotypes of Ponderosa pine (Arbaugh et al., 1998; Peterson et al., 1991; Staszak
2 et al., 2004) and Jeffery pine (Arbaugh et al., 1998; Grulke et al., 2003b; Patterson and Rundel,
3 1995; Peterson et al., 1987). Other important conifers in the region, such as giant sequoia,
4 appear to be relatively O₃-tolerant (Grulke et al., 1996). The symptoms of foliar injury and
5 growth reductions have been verified on seedlings in O₃ exposure chambers (Momen et al.,
6 2002; Momen and Helms, 1996; Temple, 1988).

7 Ozone foliar injury of dominant pine species in the Sierra Nevada Mountains is correlated
8 to decreased radial growth in both Ponderosa pine (Peterson et al., 1991) and Jeffrey pine
9 (Patterson and Rundel, 1995; Peterson et al., 1987). Because of the large amount of intraspecific

1 variation in O₃ sensitivity in these two species, O₃ exposure may be a selective agent (Patterson
2 and Rundel, 1995), with differential mortality rates for sensitive individuals (Carroll et al.,
3 2003). The region's forests may also be experiencing subtle changes in species composition and
4 community structure (Patterson and Rundel, 1995; Takemoto et al., 2001).

5 Based on fire scar dating, reconstructions of stand age classes, historical records, and
6 present stand structure, fire has been largely excluded in western forests for the last 75 to 100
7 years (Minnich, 1999; Minnich and Padgett, 2003). Fire exclusion has resulted in fewer large
8 stand-replacing fires rather than a mosaic of smaller low-intensity fires. The change in fire
9 intensity may have selectively altered stand structure, fitness and competitiveness of component
10 species, along with their susceptibility to atmospheric pollutants and other stressors (Minnich,
11 1999). Short-lived (50 to 80 years) species such as knobcone and Coulter pine (*Pinus coulteri* D.
12 Don), which occur at the interface of the chaparral and the mixed conifer forest, may already
13 have been selected for O₃ tolerance by seedling establishment (the most sensitive tree age class
14 in conifers) after large fires in the 1950s (Minnich, 1999). Strong measures to suppress fires
15 have largely kept chaparral fires from invading the mixed conifer forests, and stand densification
16 in the mixed conifer zone has increased. High stand density, in turn, may weaken the younger
17 cohorts and increase sensitivity to atmospheric pollution (Minnich, 1999).

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

1 **AX9.6.2.4 Appalachian Mountains**

2 The southern Appalachian Mountain region experiences some of the highest O₃ exposures
3 of any natural areas in the eastern United States (Chappelka et al., 1997; Hildebrand et al., 1996;
4 Mueller, 1994; Samuelson and Kelly, 1997). Since the region is the home of the Shenandoah
5 and Great Smoky Mountains NPs, which have Class I air quality designations by the 1977
6 Clean Air Act, there has been considerable study of the region's dominant forest species to
7 determine O₃ effects. Visible foliar symptoms of O₃ have been found in natural ecosystems
8 consisting of sassafras (*Sassafras albidum*) (Chappelka et al., 1999a), black cherry (*Prunus*
9 *serotina*) (Chappelka et al., 1997; 1999c; Hildebrand et al., 1996; Samuelson and Kelly, 1997),
10 yellow poplar and white ash (*Fraxinus americana*) (Chappelka et al., 1999a; Hildebrand et al.,
11 1996). Visible foliar symptoms induced by O₃ have been recreated on the same species in
12 chamber studies (Chappelka et al., 1985; Chappelka and Chevone, 1986; Duchelle et al., 1982;
13 Fredericksen et al., 1995; Samuelson, 1994). No response to O₃ exposure has been found for
14 other hardwood trees, nor for the three conifer species tested (Neufeld et al., 2000).

15 Long-term foliar injury symptoms have been correlated with decreased radial growth in
16 yellow poplar and black cherry (Somers et al., 1998) and with decreased biomass in cherry
17 (Neufeld et al., 1995). Although climatic conditions (drought) largely explained radial growth
18 reductions, O₃ exposure may have also contributed (McLaughlin and Downing, 1996). Ozone
19 exposure may also be affecting the understory vegetation in the region (Chappelka et al., 1997;
20 2003; Davison et al., 2003; Duchelle et al., 1983; Duchelle and Skelly, 1981) and community
21 composition (Barbo et al., 1998), through impacts on both growth and reproduction (Chappelka,
22 2002). Foliar litter from trees exposed to elevated O₃ have lower decomposition rates (Kim
23 et al., 1998). Other air pollutants are likely to be found in this ecosystem but not at the high
24 deposition values found in the California studies. A decline in forest health in the northern
25 Appalachians has been primarily attributed to the effects of acidic fog and rain on soil
26 acidification, lower Ca²⁺ availability, reduction in fine root biomass, and modification of
27 cuticular wax. However, fog- and O₃-exposed red spruce forests in New England also show
28 winter injury (Percy et al., 1992).

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

2 One of the most well-documented studies of population and community response to O₃
3 effects are the long-term studies of sunflower (*Plantago major*) in native plant communities in
4 the United Kingdom (Davison and Reiling, 1995; Lyons et al., 1997; Reiling and Davison,
5 1992c). Sensitive populations of *P. major* had significant growth decreases in elevated O₃
6 (Pearson et al., 1996; Reiling and Davison, 1992a,b; Whitfield et al., 1997) and reduced fitness
7 as determined by decreased reproductive success (Pearson et al., 1996; Reiling and Davison,
8 1992a). While spatial comparisons of population responses to O₃ are complicated by other
9 environmental factors, rapid changes in O₃ resistance were imposed by ambient levels and
10 variations in O₃ exposure (Davison and Reiling, 1995). Molecular patterns of genetic variation
11 suggest that a change in O₃ resistance over time probably resulted from natural selection in
12 genotypes already present in local populations, rather than through an influx of new *P. major*
13 germplasm (Wolff et al., 2000). At the site of sunflower seed collection the highest correlations
14 occurred between O₃ resistance and ambient O₃ concentrations occurred (Lyons et al., 1997),
15 rather than between O₃ resistance and other climatic variables, as found for aspen (Berrang et al.,
16 1991).

17 18 **AX9.6.2.6 Forest Health in the Carpathian Mountains**

19 The Carpathian Mountains cross five countries (the Czech Republic, the Slovak Republic,
20 Poland, Romania, and the Ukraine) and contain many national parks and several biosphere
21 reserves. The forests were largely cleared in the 15th century and were replanted with Norway
22 spruce. As elevation increases, beech (*Fagus sylvatica*) or beech-fir (*Abies alba*) forests grade
23 into Norway spruce or spruce-fir forests. Near the treeline, Norway spruce mixes with dwarf
24 mountain pine (*Pinus mugo*). Dwarf mountain pine forms an almost pure stand just below the
25 alpine vegetation.

26 The forests of the Carpathian Mountains have been subjected to anthropogenic stressors
27 (e.g., shepherding, metal mining, wood harvest for structures and paper) for hundreds of years,
28 as described for the Tatra Mountains in the southern Carpathians (Wezyk and Guzik, 2002). The
29 Carpathians have been subjected to regional air pollution stressors since industrialization. Most
30 of the effects of air pollution on forest health degradation were due to (1) heavy metal
31 deposition, (2) soil acidification by acid deposition, and (3) subsequent pest outbreaks, the

1 combination of which led to the forest decline and dieback between 1970 and 1989 (Dunajski,
2 2002). Industrial pollutants such as SO₂ and heavy metals have significantly declined since the
3 1980s, but O₃ exposure has continued to increase (Bytnerowicz et al., 2002a; Bytnerowicz et al.,
4 2004). The increased ownership and use of private cars in Central Europe, as well as the
5 long-range transport of O₃ from western Europe, are believed to be responsible for the continued
6 increase in photooxidants. In 1995, drought resulted in significant forest mortality, as well as an
7 epidemic of bark beetle infestation in subsequent years. A network of air quality monitoring
8 sites was installed across Europe in the late 1980s as part of the International Cooperative
9 Programme on Assessment and Monitoring of Air Pollutant Effects on Forests (ICP Forests).
10 Mean defoliation rates for six important forest species across Europe have increased or remained
11 unchanged from 1989 to 1999 (Percy et al., 2002). Ozone concentrations experienced in the
12 Tatra Mountains, especially along the southern slopes, occasionally reach 190 to 200 ppb as
13 2-week-long averages, with the highest values experienced in early summer at elevations of
14 1700 to 2300 m (Bytnerowicz et al., 2004). In other parts of the Carpathian Mountains, peak
15 2-week average O₃ concentrations were lower, at 160 ppb (Bytnerowicz et al., 2002b). For all
16 trees inventoried, about 13% exhibited greater than 25% defoliation during 1997 to 2000. There
17 was no difference between extent of damage for broadleaves or conifers. Trees in Poland and
18 the Czech Republic were the most affected by air pollution, while the least damaged forests were
19 in Romania (Bytnerowicz et al., 2002b).

20 The extent to which O₃ exposure affects forest health degradation, and slows forest
21 degradation, is still unknown in Europe. In many of the published studies, the response to a
22 known O₃ gradient is largely confounded by other pollutants and/or climatic gradients (Szaro
23 et al., 2002; Widacki et al., 2002). Current levels of ambient O₃ are believed to be high enough
24 to reduce bole radial growth (Percy et al., 2002). Although average O₃ concentration alone was
25 not related to bole growth, the peak hourly O₃ concentration was negatively correlated to bole
26 growth (Muzika et al., 2004). Recent evidence indicates that canopy health of European white
27 oak (*Quercus robur*), Norway spruce, maritime pine (*Pinus pinaster*), and beech has
28 significantly declined (Huttunen et al., 2001). However, the canopy health of Scots pine has
29 improved. The network of air quality monitoring stations and forest plots is extensive and
30 active. Subsequent correlative analyses including both meteorological and air quality attributes

1 throughout the European Union (EU) will help to determine the specific role of O₃ exposure in
2 forest decline. Historical effects of anthropogenic disturbance may still be confounding.

3 4 **AX9.6.2.7 Field Exposure System (FACE), Rhinelander, Wisconsin**

5 The Aspen Free-Air CO₂ Enrichment facility was designed to examine the effects of both
6 elevated CO₂ and O₃ on aspen (*Populus tremuloides*), birch (*Betula papyrifera*), and sugar maple
7 in a simple reconstructed plantation characteristic of Great Lakes Aspen-dominated forests
8 (Karnosky et al., 2003b; Karnosky et al., 1999). Instead of using chambers to expose the plants
9 to desired gas concentrations, the gas is piped up vertical delivery tubes in the open air. The
10 vertical delivery pipes surround a 30-m diameter circular plot with five different aspen clones in
11 half of the plot, one quarter of the plot planted in aspen and birch, and one quarter in aspen and
12 maple. The O₃ treatment for the first 5 years was 1.5× ambient, with ambient O₃ exposures
13 averaging 35 to 37 ppb (12 h daytime average over the growing season) compared to elevated O₃
14 rings averaging 49 to 55 ppb for the same time period (Karnosky et al., 2003b).

15 Elevated CO₂, elevated O₃, and elevated CO₂ + O₃ have had effects on most system
16 components being measured in the study (Table AX9-26) (Karnosky et al., 2003b). One
17 interesting finding of the project has been the nearly complete offset by elevated O₃ of the
18 enhancements induced by elevated atmospheric CO₂ for the pioneer keystone species aspen
19 (Isebrands et al., 2001) and birch (Percy et al., 2002) even though O₃ exposure alone did not
20 always result in a significant response when compared to controls. They also found evidence
21 that the effects on above- and below-ground growth and physiological processes have cascaded
22 through the ecosystem, even affecting microbial communities (Larson et al., 2002; Phillips et al.,
23 2002). This study also confirmed earlier observations of changes in trophic interactions
24 involving keystone tree species, as well as important insect pests and their natural enemies
25 (Table AX9-26) (Awmack et al., 2003; Holton et al., 2003; Percy et al., 2002).

26 27 **AX9.6.3 Landscape Condition**

28 In the SAB framework (Figure AX9-20), landscape condition is assessed using the areal
29 extent, composition of component landscape ecosystems or habitat types, and the pattern or
30 structure of component ecosystems or habitat types (including biocorridors). To date, no
31 publications exist on the impacts of O₃ exposure on landscape condition. The effects of O₃

Table AX9-26. Summary of Responses of *Populus tremuloides* to Elevated CO₂ (+200 μmol mol⁻¹), O₃ (1.5 × ambient), or CO₂+O₃ Compared with Control During 3 Years of Treatments at the Aspen FACE Project (Modified from Karnosky et al. (2003b))

	CO ₂	O ₃	CO ₂ + O ₃	Reference
<i>Foliar Gene Expression and Biochemistry</i>				
Rubisco; RbcS ² transcripts	d ¹	d	dd	Noormets et al. (2001a); Wustman et al. (2001)
PAL transcripts	d	I	d	Wustman et al. (2001)
Acc oxidase, catalase	d	I	d	Wustman et al. (2001)
Ascorbate peroxidase	d	n.s.	d	Wustman et al. (2001)
Glutathione reductase	d	I	d	Wustman et al. (2001)
Phenolic glycosides	I	d	n.s.	Kopper and Lindroth (2003a,b); Lindroth et al. (2001)
Tannins	n.s.	i	i	Kopper and Lindroth (2003a,b); Lindroth et al. (2001)
Foliar nitrogen	d	n.s.	d	Kopper and Lindroth (2003a,b); Lindroth et al. (2001)
C:N ratio of foliage	i	n.s.	ii	Lindroth et al. (2001)
Starch	d	d	n.s.	Wustman et al. (2001)
<i>Gas Exchange</i>				
A _{max} lower canopy	n.s.	dd	id	Noormets et al. (2001b); Takeuchi et al. (2001)
A _{max} whole canopy	ii	dd	n.s.	Noormets et al. (2001a); Sharma et al. (2003)
Stomatal limitation	d	n.s.	d	Noormets et al. (2001b)
Stomatal conductance	d	di	d	Noormets et al. (2001b)
Foliar respiration	n.s.	i	n.s.	Noormets et al. (2001a); Takeuchi et al. (2001)
Soil respiration	ii	d	n.s.	King et al. (2001)
Microbial respiration	ii	n.s.	n.s.	Phillips et al. (2002)
Stomatal density	n.s.	n.s.	n.s.	Percy et al. (2002)
Chlorophyll content	d	d	d	Wustman et al. (2001)
Chloroplast structure	i	d	d	Oksanen et al. (2001); Takeuchi et al. (2001); Wustman et al. (2001)
O ₃ flux	d	ii	i	Noormets et al. (2001b)
<i>Growth and Productivity</i>				
Leaf thickness	i	n.s.	n.s.	Oksanen et al. (2001)
Leaf size	i	d	d	Wustman et al. (2001)
Leaf area	i	d	n.s.	Noormets et al. (2001a)

¹Responses 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.).

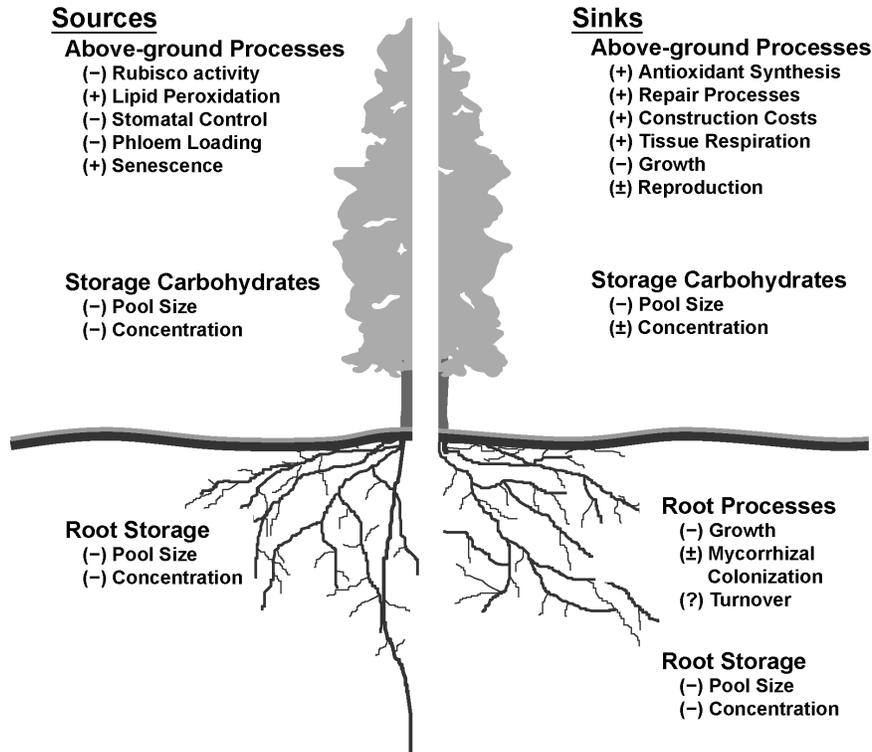


Figure AX9-20. A conceptual diagram of processes and storage pools in sources and sinks that are affected by O_3 exposure. A plus (+) denotes an increase in process rate or pool size, a minus (-) denotes a decrease in process rate or pool size, and a plus-minus (\pm) denotes that both increases and decreases 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).

1 exposure have only been reported at the community or stand level (see Biotic Conditions,
 2 below). The following is a description of current discussions by land stewards and of how
 3 difficult it will be to quantitatively assess the effect of O_3 exposure on landscape condition.

4 Landscapes are identified and preserved, such as national parks, Class I wilderness areas,
 5 etc., so that they are protected from the effects of O_3 exposure by law. Efforts to determine
 6 whether landscapes have been affected by certain levels of exposure rely on valuation of
 7 landscape and ecosystem components. Several different approaches of valuation have been
 8 used, including pathological (visible symptoms), biomass and allocation, and biogeochemical.

1 In the pathological approach, a “critical levels” concept is developed, with varying levels
2 of impact viewed as acceptable, interim targets, or as unacceptable. As an example, land
3 managers of Class I wilderness areas may consider a level acceptable if it resulted in no
4 visible O₃ symptoms to sensitive species. In concrete terms, sensitive species may respond to
5 peak O₃ exposures of 60 ppb (e.g., coneflower, in Great Smoky National Mountains NP;
6 [Davison et al., 2003]), and so the critical exposure level would be < 60 ppb for any hourly
7 valued during the growing season. An interim target would be that less than 5% of the sensitive
8 plants would have visible symptoms on <15% of the leaf surface. An unacceptable level of O₃
9 exposure would be any result more pronounced than the interim target. The advantage of the
10 foliar injury approach is that large crews with relatively simple training can assess individual
11 species within the landscape and “see” the effect of the oxidant exposure. There are several
12 disadvantages, however. Some species (e.g., white fir) exhibit no foliar injury but do have shifts
13 in biomass allocation in response to oxidant exposure (Retzlaff et al., 2000). Other species have
14 shown significant decreases in foliar injury due to needle loss, with retranslocation of nutrients
15 to remaining foliage, and subsequent increased photosynthetic rate (Beyers et al., 1992).
16 In addition, the development of foliar symptoms within a species is related to sunlight and
17 microclimate (Davison et al., 2003).

18 In the biomass approach, O₃ exposure resulting in a measurable decline in biomass (usually
19 of a target, sensitive species) is used to evaluate landscape condition. The bulk of the
20 information available is from seedling responses to controlled chamber exposures, reviewed in
21 the previous section. Some information exists for species in natural environments, but teasing
22 out concurrent stressors and finding adequate controls may be intractable. For example, in a
23 long-term gradient of O₃ exposure, N deposition, and drought, the site with the highest O₃
24 exposure had the greatest whole tree biomass (pole-sized trees) due to growth stimulation by N
25 deposition (Grulke and Balduman, 1999).

26 In the biogeochemical approach, changes in biogeochemical cycling are used to assess
27 landscape condition. O₃-sensitive species have well known responses to O₃ exposure, including
28 altered C allocation to below- and above-ground tissues, and altered rates of leaf production,
29 turnover, and decomposition. Changes in turnover rates of ephemeral tissues (leaves, fine roots)
30 also affect nutritional status of the remaining tissue. These shifts can affect overall C and N loss
31 from the ecosystem in terms of respired C, and leached aqueous dissolved organic and inorganic

1 C and N. Instability in C and N pools and their dynamics can affect landscape-level nutrient
2 dynamics even without significant inputs of N deposition. The endpoint assessment is based on
3 changes in water quality from or in the landscape, correlated to a defined oxidant exposure level.
4 These approaches are linkable: visible injury at a particular level could be related to reduction in
5 photosynthate, which would reduce whole plant biomass (and carbon dynamics). If O₃-sensitive
6 species dominate the landscape, then changes in C and N dynamics over time would be expected
7 to alter biogeochemical cycles. Examples of forest types that contain geographically extensive,
8 O₃-sensitive species that could be used in assessing landscape-level changes include Ponderosa
9 pine in the western United States, yellow poplar or loblolly pine in the eastern United States
10 deciduous forests, and Norway spruce in the Carpathian Mountains of eastern Europe.

11 Water quantity may also be affected by O₃ exposure at the landscape level. Moderately
12 high O₃ exposure may affect the mechanism of stomatal opening (McAinsh et al., 2002),
13 resulting in sluggish stomatal opening and closing (Reich and Lassoie, 1984). During
14 moderately high O₃ exposure in a drought year, canopy transpiration was greater for yellow
15 poplar than on adjacent days with lower O₃ exposure, which could alter water use at the
16 landscape level. Oxidant exposure (O₃ and NO_x) may decrease the ability of exposed plants to
17 close stomata at night (Grulke et al., 2004), thus increasing water loss from the landscape.
18 Ecosystem models should aid in interpreting O₃-exposure effects at the landscape level.

20 **AX9.6.4 Biotic Condition**

21 **AX9.6.4.1 Ecosystems and Communities**

22 The SAB framework described by Young and Sanzone (2002) identifies community extent,
23 community composition, trophic structure, community dynamics, and physical structure as EEAs
24 for assessing ecosystem health.

26 **COMMUNITY EXTENT**

27 Ecosystem function is dependent on areal extent, constituent species composition, trophic
28 structure and its dynamics, and community physical structure. Genetic variation within species,
29 and the dynamics of the interactions that exist among different species and their biotic and
30 abiotic environment, are also involved (Agrawal and Agrawal, 2000). There are no reports of O₃
31 exposure altering community distribution or extent.

1 COMMUNITY COMPOSITION

2 Significant changes in plant community composition resulting directly from O₃ exposure
3 has been demonstrated in two forested areas: the mixed conifer forest of the San Bernardino
4 Mountains, CA and the mixed conifer forest of the Valley of Mexico near Mexico City. It is
5 also likely that community composition has changed in response to O₃ exposure in the
6 coniferous forests of the Carpathian Mountains, but this has not yet been definitively shown.

7 The first forest communities shown to be affected by O₃ were the *Pinus ponderosa*-
8 dominated stands of the San Bernardino Mountains in southern California (Miller, 1973). Miller
9 suggested that mixed forests of *P. ponderosa*, *Pinus jeffreyi*, and *Abies concolor* were changing
10 to predominantly *A. concolor* because of the greater O₃ sensitivity of the pines. Significantly
11 greater mortality of young mature trees (50 to 99 years old) occurred in sites that also showed
12 higher foliar injury relative to sites that showed slight foliar injury (McBride and Laven, 1999).
13 For *P. ponderosa*, 33% of the trees in the high foliar injury sites died versus 7% of the trees in
14 the low foliar injury sites over the decade-long census. In contrast, 24% of *Abies concolor* died
15 in high foliar injury sites, whereas no trees died in slight injury sites. The authors suggested that
16 certain age classes were especially sensitive to O₃ exposure, because they are emerging into the
17 canopy where higher O₃ concentrations are encountered. Future projections based on past
18 changes in community composition have been conducted for 2024 and 2074 (McBride and
19 Laven, 1999). In their projections, the population of Ponderosa pine nearly disappears in all tree
20 age classes, and the community is dominated by California black oak (*Quercus kelloggii*) in all
21 tree age classes, followed by Incense-cedar (*Calocedrus decurrens*) and sugar pine (*Pinus*
22 *lambertiana*) by the year 2074. Their projections do not account for potential changes in genetic
23 structure of the more O₃-sensitive species.

24 In the Valley of Mexico, the closed forest structure changed to a woodland from high
25 pollutant exposure (Fiscus et al., 2002). Cryptogamic community diversity also significantly
26 declined in response to prolonged, extreme O₃ exposure (Zambrano and Nash, 2000). Together,
27 these two examples illustrate the potential for shifts in community composition in response to O₃
28 stress.

29

1 **TROPHIC STRUCTURE**

2 *Above-Ground*

3 One of the first reports of trophic level interactions in natural communities was
4 the O₃-induced predisposition of Ponderosa pine to attack by bark beetles (Cobb et al.,
5 1968; Stark et al., 1968; Stark and Cobb, 1969). Trees exposed to oxidant injury had lower resin
6 production, flow, and exudation pressure. Also, several attributes associated with tree defense
7 against beetle attack including sapwood and phloem moisture content and phloem thickness
8 were compromised by oxidant exposure (Pronos et al., 1999). Another trophic level has been
9 implicated, in that O₃-injured Ponderosa pine had the same rate of bark beetle infection, but
10 healthy trees had greater numbers of bark beetle predators and parasitoids (Dahlsten et al., 1997).
11 This suggests that O₃ damage rendered the pines inhospitable for the natural enemies of the bark
12 beetles. Similar findings were presented by Percy et al. (2002) for aphids whose abundance was
13 increased in young *Populus tremuloides* stands exposed to elevated O₃. In that study, the levels
14 of natural enemies of aphids (ladybirds, lacewings, spiders, and parasitoids) were significantly
15 decreased under elevated O₃.

16 17 *Below-Ground*

18 Processing of plant-derived carbon compounds by soil organisms comprising the soil food
19 web is a fundamental property of a functional and stable below-ground ecosystem (De Ruiter
20 et al., 1998; Wolters, 1998). Soil food web organisms are responsible for recycling nutrients and
21 for development of soil properties such as porosity, aggregate structure, water-holding capacity,
22 and cation exchange capacity. A shift in food-web species diversity or functional complexity in
23 response to O₃ stress may alter ecosystem processes.

24 Evidence that soil organisms are affected by O₃ indicates the potential for changes in soil
25 food-web structure and function. Since O₃ does not penetrate the soil beyond a few centimeters,
26 the proposed mechanism by which O₃ alters soil biota is through a change in C input to soils
27 (Andersen, 2003). Ozone can alter C inputs to soil and hence soil processes through four
28 different pathways: (1) leaf-litter quality and quantity (see Material Cycling, below), (2) C
29 allocation to roots (see Physiological Status, below), (3) interactions among root symbionts, and
30 (4) rhizodeposition. The complex nature of the effects of O₃ on trophic interactions and food
31 webs calls for additional basic research and modeling.

1 There have been no comprehensive studies on the effects of O₃ on structural components of
2 soil food webs; however, studies have shown that O₃ affects free-living soil organisms of food
3 webs. In the few cases where soil microbial communities have been examined, O₃ has led to
4 changes in bacterial and fungal biomass, and, in some cases, changes in soil enzyme activity.
5 Phillips et al. (2002) examined the effects of elevated CO₂ and O₃ on C flow through
6 heterotrophic microbial communities in soils collected from a FACE study in Wisconsin. Ozone
7 decreased abundance of fungal phospholipid fatty acids in aspen and birch-aspen plots but had
8 few other direct effects on measured soil parameters. The greatest effect of O₃ was to eliminate
9 significant increases in microbial respiration resulting from elevated CO₂, suggesting an
10 important role for O₃ in altering C flow through soils. Shafer (1988) found that O₃ tended to
11 increase the number of fungal propagules and bacteria exhibiting phosphatase activity in the
12 rhizosphere of sorghum. Ozone in combination with simulated acid rain stimulated soil
13 arylsulfatase activity (Reddy et al., 1991). The response was observed at low concentrations, but
14 was reversed at high concentrations, suggesting a threshold level of O₃, possibly involving
15 different mechanisms. Ozone significantly decreased soil microbial biomass in the fall after one
16 season of exposure in a wheat and soybean system (Islam et al., 2000). Other studies have
17 shown shifts in microbial and fungal biomass in response to O₃ stress, but responses were
18 variable (Scagel and Andersen, 1997; Yoshida et al., 2001).

19 Decreased allocation to roots associated with O₃ exposure alters N fixation in legumes and
20 actinorrhizal species. Ozone exposure was found to decrease nodulation in a number of species
21 (Manning et al., 1971; Tingey and Blum, 1973). In alder (*Alnus serrulata*), host root cells of
22 nodules showed cytoplasmic breakdown and lacked organelles when seedlings were exposed
23 to O₃ for 27 days (Greitner and Winner, 1989).

24 Ozone has been shown to affect mycorrhizal colonization (Adams and O'Neill, 1991;
25 Edwards and Kelly, 1992; Ho and Trappe, 1984; McCool et al., 1982; Simmons and Kelly, 1989;
26 Smith and Read, 1997). Although short term in nature, several studies have found enhanced
27 mycorrhizal short-root formation under O₃ stress. White pine (*Pinus strobus*) (Stroo et al.,
28 1988), Norway spruce (Rantanen et al., 1994), Northern red oak (*Quercus rubra*) (Reich et al.,
29 1985), Douglas fir (*Pseudotsuga menziesii*) (Gorissen et al., 1991a), European silver fir (*Abies*
30 *alba*) (Wollmer and Kottke, 1990), and Scots pine (Kasurinen et al., 1999) all showed some
31 increase in mycorrhizal presence when exposed to O₃. Others have shown minimal or no effects

1 of O₃ on mycorrhizas (Kainulainen et al., 2000; Mahoney et al., 1985; Meier et al., 1990). Stroo
2 et al. (1988) found that percent infection increased from 0.02 to 0.06 ppm O₃, then decreased
3 from 0.06 to 0.14 ppm; the total number of short roots were unaffected, however. In cases where
4 stimulation was observed, the response was often noted shortly after initiation of exposure, often
5 at relatively low concentrations. Good examples of this transitory response can be found in
6 results with Norway spruce and Scots pine (Kasurinen et al., 1999; Rantanen et al., 1994). In
7 these studies, O₃ increased mycorrhizal short roots initially but differences were not evident by
8 the end of the experiment.

9 Evidence suggests that decreased below-ground allocation associated with O₃ stress alters
10 mycorrhizal host-symbiont compatibility. Edwards and Kelly (1992) found a shift in fungal
11 morphotypes present on loblolly pine roots, even though the number of mycorrhizal short roots
12 per gram fine root was not significantly affected by O₃. Qui et al. (1993) found increased
13 numbers of morphotypes present in O₃-sensitive loblolly pine seedlings exposed to O₃. Roth and
14 Fahey (1998) found an interaction between O₃ and acid precipitation treatments on the
15 composition of fungi forming ectomycorrhizae on red spruce saplings, possibly driven by
16 nutrient availability. Carbohydrate requirements vary among fungal species (Bidartondo et al.,
17 2001), and O₃ may affect species composition by altering carbohydrate availability in roots.
18 A shift in species dominance could lead to a change in successional patterns of mycorrhizal
19 communities.

20 In the few studies that examined root exudation in response to O₃ exposure, O₃ was found
21 to alter the quantity and quality of root exudates. McCool and Menge (1983) found a significant
22 decrease in exudation of amino acids in tomato exposed to 300 ppb O₃. McCrady and Andersen
23 (2000) observed increased root exudation in nonmycorrhizal wheat. No apparent change in root
24 exudation was found in labeling studies of ECM Ponderosa pine (Andersen and Rygielwicz,
25 1995a). Inconsistency in the literature probably results from species differences and
26 experimental protocols; however, these examples illustrate the potential effects of O₃ on
27 rhizosphere C flux.

28 Decreased C allocation to roots of O₃-exposed plants may reduce root longevity and
29 accelerate root turnover, increasing rhizodeposition of C and N. Fine root turnover decreased in
30 mature northern red oak exposed to elevated O₃ (seasonal exposure ranging from 152 to
31 189 ppm-h), whereas seedlings did not show any reduction in turnover (Kelting et al., 1995).

1 King et al. (2001) found a trend toward decreased live root biomass and increased dead root
2 biomass in aspen exposed to O₃ in a FACE study, suggesting possible changes in both
3 production and longevity.

4 Other studies also suggest that O₃ alters C flux to soils, resulting in changes in CO₂ efflux
5 from soils. Both root respiration and soil CO₂ efflux decreased from loblolly pine seedlings
6 exposed to O₃ (Edwards, 1991). Soil CO₂ efflux increased in response to O₃ in Ponderosa pine
7 seedlings (Andersen and Scagel, 1997). No direct assessments of hyphal growth and turnover in
8 response to O₃ stress have been conducted. Ozone decreased C allocation to extrametrical
9 hyphae of a Ponderosa pine mycorrhiza, which might be expected to decrease growth and
10 increase hyphal turnover (Andersen and Rygiel, 1995b).

11 12 **COMMUNITY DYNAMICS, PHYSICAL STRUCTURE**

13 One of the best-documented examples of change in long-term forest community dynamics,
14 of dominant overstory trees occurred in the San Bernardino Mountains between 1968 and 1974
15 (reported in Miller (1973) and Miller et al. (1989)). Plots were recently re-inventoried ~25 years
16 after establishment (Arbaugh et al., 2003). Of the six codominant canopy species, white fir
17 showed the greatest change, increasing in both numbers and bole growth for a 286% change in
18 basal area/ha in the San Bernardino Mountains. Sugar pine basal area also increased
19 significantly (by 334%), but this species represents only a small portion (1%) of the total basal
20 area of the forest sampled. The most O₃-sensitive species (Miller et al., 1982), Ponderosa and
21 Jeffrey pine, had the lowest increase in basal area/ha (76 and 62%, respectively). These two
22 species represented 72% of the basal area/ha of all stands inventoried. Ponderosa pine had the
23 greatest mortality rate of all canopy species inventoried (46%), followed by white fir and black
24 oak (35% and 33%), Jeffrey pine (29%) and Incense cedar and sugar pine (both 7%). In moist
25 sites (at the western end of the San Bernardino Mountains), there was significant recruitment of
26 Incense cedar, white fir, and sugar pine. Only one study directly attributed tree mortality to O₃
27 exposure: it accounted for 7% of mortality in the Sierra and Sequoia NFs (Carroll et al., 2003).

28 Species diversity in the understory can be quite large, making studies of O₃ effects on
29 understory community dynamics very challenging. However, there have been some attempts to
30 quantify understory responses, ranging from describing relative sensitivity to their visible
31 symptoms (Temple, 1999; Treshow and Stewart, 1973) to very complex measures of community

1 structure and composition (Westman, 1979, 1981). The lowest percentage cover and lowest
2 species diversity in California coastal sage scrub was correlated with the highest O₃ exposures as
3 estimated by extrapolation from the closest air monitoring stations (Westman, 1979). The
4 understory also has the potential to influence responses to O₃ of dominant keystone species, as
5 has been shown in controlled experiments with both Ponderosa pine (Andersen et al., 2001) and
6 Loblolly pine (Barbo et al., 2002). Barbo et al. (1998) exposed an early successional forest
7 community to ambient air, charcoal-filtered air, non-filtered air, and 2× ambient in the
8 Shenandoah NP. They found changes in species performance, canopy structure, species
9 richness, and diversity index consistent with the view that O₃ can induce a shift in vegetation
10 dominance and community structure.

11 There have been few studies evaluating the effect of O₃ exposure on the physical structure
12 of natural ecosystems. Despite an extensive array of allometric equations for conifers in the
13 western United States (Ter-Mikaelian and Korzukhin, 1997), none appear to predict individual
14 tree shape in a site of moderate O₃ exposure, suggesting that O₃ may effect allometry (Grulke
15 et al., 2003a). Canopy structural changes are also implied by the measure of canopy
16 transparency used in the USDA Forest Service's Forest Health Monitoring (FHM) assessment.
17 The loss of epiphytic lichens within the canopy is a clear example of plant community structural
18 change occurring along an O₃ gradient (Nash and Sigal, 1999; Zambrano and Nash, 2000).

19 As of yet, there have been no comprehensive studies on the effects of O₃ on structural or
20 functional below-ground components (Andersen, 2003). Phillips et al. (2002) found evidence
21 for changes in the bacterial and fungal biomass below aspen and aspen/paper birch stands
22 exposed to elevated O₃. Subsequent study showed that O₃ exposure decreased cellobiohydrolase
23 activity in the soil microorganisms, driving the change in the microbial community (Larson
24 et al., 2002).

25 26 **AX9.6.4.2 Species and Populations**

27 Ozone can affect species and populations of species found in ecosystems through changes
28 in population size, genetic diversity, population structure and/or dynamics, and habitat suitability
29 (Young and Sanzone, 2002). For example, if individuals of a species are lost due to O₃
30 exposure, population size declines. Often very young (e.g., conifer seedlings, see Section
31 AX9.6.4.3 below) and old individuals differ in their sensitivity, so that population structure also

1 will be altered by O₃ exposure. If resource allocation to reproductive output is altered by O₃
2 exposure, population dynamics will be altered. Communities dominated by O₃ sensitive species
3 in the canopy or understory may be altered sufficiently for the habitat to become unsuitable for
4 other species. Genetic selection acts on the individual plant, which represents a certain
5 proportion of the populations' genetic variation. If an O₃- sensitive individual succumbs
6 through multiple stresses, including O₃ stress, the genetic variation represented in the population
7 generally declines, unless sensitive individuals have low inherent genetic variability (e.g.,
8 Staszak et al., 2004).

9 While the concept of natural selection induced by O₃ exposure and related changes in
10 natural plant communities has been around for a long time (Dunn, 1959; Miller et al., 1972), the
11 concept of evolution of O₃ tolerance is still not widely accepted. The unequivocal demonstration
12 that considerable genetic variation in O₃ resistance exists within and between plant populations,
13 and that ambient levels of O₃ may differentially affect fitness-related traits (i.e., growth, survival,
14 and fecundity), suggests that O₃ may potentially drive the natural selection for resistant
15 individuals. Dunn (1959) presented circumstantial evidence that ambient O₃ in the Los Angeles
16 area was high enough to drive the selection of O₃-resistant *Lupinus bicolor* genotypes. Since
17 Dunn's (Dunn, 1959) research on O₃-induced population changes, researchers have
18 demonstrated differences in O₃ tolerance among other plant populations. In the devastating
19 forest decline southeast of Mexico City, the remaining trees (primarily *Abies religiosa* in the
20 "cemetery forests") appear to be less affected by foliar injury than trees that were lost, despite
21 continued high-O₃ exposures (Alvarado et al., 1993). However, even the most convincing work
22 in this field (Berrang et al., 1986, 1989, 1991), with *Populus tremuloides*, where a strong
23 correlation between visible foliar injury after O₃ exposure and maximum O₃ concentration at the
24 origin of the population was shown (Berrang et al., 1991), a change in gene frequency at any one
25 site over time has not yet been demonstrated (Bell et al., 1991; Reiling and Davison, 1992a).
26 Furthermore, the selection intensity of O₃ has been questioned (Bell et al., 1991; Taylor et al.,
27 1994; Taylor and Pitelka, 1992) and the emergence of O₃ exposure since the 1950s as an
28 environmental stressor may not have been long enough to affect tree populations with long
29 generation times (Barrett and Bush, 1991).

30 The loss of O₃-sensitive individuals results in natural selection favoring O₃-tolerant species
31 (Bradshaw and McNeilly, 1991). Increased levels of mortality of O₃-sensitive individuals have

1 occurred for *Pinus jeffreyi* and *P. ponderosa* exposed to ambient O₃ along the western slope of
2 the Sierra Nevadas (Miller et al., 1998; Peterson et al., 1987), for *Pinus strobus* exposed to
3 ambient O₃ in southern Wisconsin (Karnosky, 1981), and for *P. ponderosa* in the San Bernardino
4 Mountains (Carroll et al., 2003). In these examples, individuals that consistently had greater
5 foliar injury and lower needle retention were lost in repeated surveys. Ozone-induced loss of all
6 individuals except the most tolerant and breeding among the surviving individuals to yield more
7 more tolerant populations has not yet been demonstrated for plants exposed to O₃, except for the
8 relatively short-term (2 years) adaptation exhibited in *Trifolium repens* (Heagle et al., 1991) and
9 *Plantago major* (Davison and Reiling, 1995). Heagle et al. (1991) were able to show the
10 adaptation of a *Trifolium repens* population to elevated O₃ in just two growing seasons.
11 Similarly, Davison and Reiling (1995) compared the O₃ resistance of *P. major* populations
12 grown from seed collected from the same sites over a period of increasing O₃. The two
13 independent populations studied exhibited increased O₃ resistance, consistent with the idea of
14 selection for O₃ tolerance. Using random amplified polymorphic DNA primers, this team also
15 showed that the later populations are subsets of the earlier ones, consistent with in-situ evolution
16 rather than with catastrophic loss and replacement of the populations (Wolff et al., 2000). The
17 problem is determining whether spatial patterns in O₃ resistance and changes in time are casually
18 related to O₃, because there were very strong correlations with other factors (Davison and
19 Barnes, 1998; Reiling and Davison, 1992c). The potential for the evolution of O₃ resistance has
20 been clearly demonstrated by Whitfield et al. (1997) in their study of O₃ selection of common
21 plantain, where they showed that within a matter of a few generations, it was possible to
22 increase O₃ resistance in an initially O₃-sensitive population. Wild radish (*Raphanus sativus*)
23 developed O₃ resistance after only one generation of exposure to O₃ (Gillespie and Winner,
24 1989).

25 A third independent line of research suggesting O₃ may be affecting the genetic diversity of
26 wild plant populations was presented by Paludan-Müller et al. (1999) who showed that northwest
27 European provenances of European beech were more sensitive to O₃ than were southeast
28 European provenances that had experienced higher O₃ levels. Recent research on the genetic
29 structure of 50-year-old Ponderosa pines in the San Bernardino Mountains suggests that distinct
30 differences in frequency of some alleles and genotypes occurred, with the O₃-tolerant trees being
31 more heterozygous (Staszak et al., 2004). While both of these studies were only correlational,

1 they are consistent with previous studies of this type, suggesting O₃-induced population changes.
2 Again, other environmental stressors besides O₃ exposure could have been involved in effecting
3 change within these populations.

4 Natural selection for O₃ tolerance can also be facilitated by reductions in fitness related to
5 lower seed yields of O₃-sensitive species or individuals. The impacts of O₃ on reproductive
6 development, recently reviewed by Black et al. (2000) can occur by influencing (1) age of
7 flowering, particularly in long-lived trees that often have long juvenile periods of early growth
8 without flower and seed production; (2) flower bud initiation and development; (3) pollen
9 germination and pollen tube growth; and (4) seed, fruit, or cone yields and seed quality (Table
10 AX9-24). In addition, vegetatively propagated species can have lower numbers of propagules
11 under elevated O₃ conditions (Table AX9-24).

12 Several studies suggest that reproductive structures are clearly sensitive to O₃ and that O₃
13 can affect fitness of plants by affecting either the sporophytic or gametic generations. Decreased
14 numbers of flower spikes and seed capsules per plant were found for plantain growing under
15 elevated O₃ (Lyons and Barnes, 1998; Pearson et al., 1996; Reiling and Davison, 1992a).
16 Similar responses were seen for *Brassica campestris* plants exposed to a single dose of 100 ppb
17 O₃ for 6 h. Stewart et al. (1996) and Bosac et al. (1998) reported an increase of flower bud
18 abortion for oilseed rape (*Brassica napus* L.) similarly exposed to a short duration of elevated
19 O₃. Floral initiation period can be delayed in O₃-sensitive plants, as was described for dogbane
20 (*Apocynum androsaemifolium*) grown under ambient O₃ in the eastern United States. In one of
21 the few comparisons of whole plant O₃ sensitivities with that of male gametophytes, Hormaza
22 et al. (1996) found a high correlation of relative O₃ sensitivity of pollen tube elongation with that
23 of O₃ effects on net photosynthesis and relative growth rates for 6 species of fruit trees.

24 Clearly, the concept of O₃-induced genetic change is an area that needs additional research
25 attention. Repeated collections over time from wild populations receiving high O₃ exposures to
26 examine population responses and relative sensitivity changes, the sampling of genetic diversity
27 along known O₃ gradients, and the use of modern biotechnological approaches to characterize
28 and quantify genetic diversity are useful approaches to test for O₃-induced impacts on diversity
29 in natural ecosystems.

1 **AX9.6.5 Organism Condition**

2 **PHYSIOLOGICAL STATUS**

3 The generalized effects of O₃ exposure on plants are well known, and have been reviewed
4 from several viewpoints over the last decade (Darrall, 1989; De Kok and Tausz, 2001; Heath and
5 Taylor, 1997; Matyssek et al., 1995; Pell et al., 1997; Reich, 1987; Schraudner et al., 1997; U.S.
6 Environmental Protection Agency, 1996). The topic of individual species response and
7 modification of response by other factors has been addressed thoroughly in Sections AX9.6.3
8 and AX9.6.4 of this chapter. Here, this subsection will describe the physiological changes in
9 response to O₃ that have been hypothesized to lead to changes in ecosystem structure or function.
10

11 *Above-Ground Responses*

12 The first critical step leading to O₃ response is uptake of O₃ by the leaves, leading to
13 changes in C and nutrient relations that are thought to alter plant growth and competitiveness
14 (see Section AX9.3). Ozone enters leaves through stomata, reacts with cell walls or membranes,
15 and starts a series of adverse reactions. Cuticular uptake of O₃ is believed to be negligible (Coe
16 et al., 1995; Kerstiens and Lenzian, 1989). Once inside the leaf, O₃ and its byproducts lead to
17 membrane disruption, chlorophyll breakdown, and decreased Rubisco levels (Schweizer and
18 Arndt, 1990). In turn, photosynthesis is decreased, as is stomatal conductance (Weber et al.,
19 1993). Also, O₃ often leads to increased maintenance respiration, decreased foliar nutrient
20 content, and imbalances in tissue nutrient content and retention. When photosynthetic pigments
21 have been damaged, the pigment must be fully broken down (and/or new N and Mg must be
22 taken up and transported to the leaf) for the pigment to be regenerated (Bjorkman and Demmig-
23 Adams, 1995). Ozone exposure alters within-plant priorities for resources: less C is available
24 for allocation to roots and spring regrowth, and less foliar biomass is retained. At the whole-
25 organism level, O₃ exposure decreases root mass (Grulke et al., 1998a) and radial bole growth
26 (Muzika et al., 2004; Peterson et al., 1991) with little impact on height growth. Visible
27 symptoms of O₃ injury vary between species and genotypes but often include upper leaf surface
28 stipple, chlorotic mottle, or large bifacial blotches of necrotic tissue. Premature senescence is
29 typical of almost all O₃-induced foliar damage. All of these changes can alter the plant's ability
30 to function in a broader ecosystem context.

1 The underlying mechanisms of O₃-injury response in conifers, broadleaf deciduous trees,
2 and herbaceous species are assumed to be similar. However, several differences in long-lived
3 species are important at the ecosystem level. Most of the research on O₃ effects has been
4 conducted on herbaceous species (i.e., crops). Although a number of native herbaceous species
5 have been identified as O₃-sensitive, there are no published physiological studies on the effect
6 of O₃ exposure on herbaceous or shrub species in situ. In natural ecosystems, the majority of
7 species are not annuals, unless the system is highly disturbed. Nonetheless, response of crop
8 species to elevated O₃ may be used as an analog for native annual response: phenological
9 staging is accelerated (soybeans; Booker et al. [2004]), thus “avoiding” additional O₃ exposure.

10 Conifers have roughly half the stomatal conductance of deciduous broadleaf trees (Reich,
11 1987), leading to proportionally less O₃ uptake at the same O₃ exposure level. Yet, except for
12 species of larch, individual conifer needles are longer-lived and active over a greater portion of
13 the year. Therefore, needle longevity can also work against the tree by increasing cumulative O₃
14 exposure and exposure to other stressors. Increased needle longevity is not always a
15 disadvantage, for example, conifers are physiologically active in early spring and late fall, during
16 times of lower oxidant concentrations. These periods can contribute significantly to a net
17 positive annual C balance and from the standpoint of nutrient storage, are important in reparation
18 responses to pollutants. Patterson and Rundel (1995) reported that Jeffrey pine had significant
19 stomatal opening (one third that of a typical summer day) in midwinter with snow on the ground.
20 At least pole-sized and larger trees can mitigate reductions in C acquisition due to oxidant
21 exposure in the summer with C assimilation on favorable days in the winter. The interaction of
22 environmental factors, plant phenology (the timing of growth events; birth and mortality of plant
23 parts), physiological status (nutritional or moisture status; dormant or active growth within the
24 year), and tree age (interannual differences in resource acquisition and allocation) all contribute
25 to the complexity of long-lived species (and hence ecosystem) response to O₃ exposure.

26 One widely observed response to O₃ exposure is premature leaf loss. As noted above,
27 premature leaf loss may reduce O₃ uptake during high-O₃ years, but it has several negative
28 consequences. Early leaf loss results in reduced C uptake through photosynthesis. Premature
29 needle loss also results in less N retranslocation compared to normally senescing leaves, which
30 reduces whole plant N balance (Fenn and Dunn, 1989) and carbohydrate availability for

1 overwinter storage (Grulke et al., 2001). Because of such effects accumulated over several years
2 of O₃ exposure, subsequent-year C and N reserves can be affected (Andersen et al., 1991).

3 Conversely, a series of drought years can decrease O₃ uptake, as well as reduce C and
4 nutrient acquisition, altering resource allocation to defenses (e.g., antioxidants) (Grulke et al.,
5 2003b) (or resins) against insect infestation, rendering the tree more susceptible to O₃ injury.
6 Conifers have thicker cuticles than either broadleaf deciduous or herbaceous species.
7 Continued O₃ exposure may compromise cuticular integrity (Bytnerowicz and Turunen, 1994).
8 Once cuticular integrity is breached, individual leaves (needles) are likely to be excised, thus
9 contributing generally to defoliation and reduced C acquisition.

10 With the exception of the extensive research conducted on mature tree response to O₃
11 exposure in California forests (Arbaugh et al., 1998; Grulke et al., 1996, 1998b, 2001, 2002b,
12 2003a,b, 2004; Grulke, 1999; Grulke and Balduman, 1999; Peterson et al., 1987, 1991, 1995;
13 Wieser et al., 2002), the vast majority of studies of O₃ effects on forest trees have been
14 conducted on young seedlings (Chappelka and Samuelson, 1998) and little is known about
15 acclimation to O₃ (Skärby et al., 1998). Chamber exposure studies can be used to document
16 foliar symptoms and develop response variables for the whole plant. These response variables
17 can then be field tested on mature trees using correlative analyses (e.g., Grulke and Lee [1997];
18 Grulke et al. [2003b]). Without the initial work in chamber exposure studies, field responses to
19 O₃ exposure would be difficult to verify and distinguish from other concurrent stressors.

20 Predicting mature tree responses to O₃ solely from seedling response studies is complex,
21 because seedlings or saplings do not necessarily respond to O₃ in the same way as mature trees
22 (Karnosky et al., 2003a; Norby et al., 1999). Although species dependent, O₃ has been found to
23 have stronger effects on leaf function in younger rather than older trees (Kolb and Matyssek,
24 2001). Each component physiological attribute “matures” at a different rate. Gas exchange
25 patterns differ between seedlings and mature trees. For example, leaf respiration of juvenile
26 Ponderosa pine was greater than that of mature trees (Momen et al., 1996). In the conifers
27 tested, the highest gas exchange rates (and by inference stomatal uptake of O₃) are found in
28 seedlings (e.g., in scions of red spruce, Rebbeck et al. [1993]; giant sequoia, Grulke and Miller
29 [1994]; Ponderosa pine, Grulke and Retzlaff [2001]; and Norway spruce, Wieser et al. [2002]).
30 Patterns of biomass (Grulke and Balduman, 1999) and carbohydrate allocation (Grulke et al.,
31 2001) differ between immature and mature trees. Pole-sized trees had greater reduction in root,

1 foliar, and bole carbohydrate concentrations than did old growth trees. Antioxidant defenses
2 vary with both tree age and needle age (Tegischer et al., 2002). Based on all attributes measured
3 in both Ponderosa pine and giant sequoia, the youngest tree age considered representative of
4 mature trees was 20 years old (Grulke et al., 1996; Grulke and Retzlaff, 2001). In some
5 broadleaf deciduous tree species, seedlings are more conservative, and mature trees have greater
6 gas exchange rates, as is the case for *Quercus rubra* (Edwards et al., 1994; Kelting et al., 1995;
7 Samuelson and Kelly, 1996, 1997) and *Fagus sylvatica* (Braun et al., 1999). In another
8 broadleaf deciduous tree species (black cherry), gas-exchange rates of seedlings were faster, but
9 total O₃ flux to leaves of seedlings was lower than that of mature trees due to differences in leaf
10 ontogeny (Fredericksen et al., 1995, 1996).

11 Nitrogen deposition modifies the effects of oxidant exposure through several offsetting
12 physiological mechanisms (see Section AX9.4.4). Nitrogen deposition, in wet or dry particulate
13 form, ultimately increases site fertility, but increased soil N availability decreases C allocation to
14 roots, further exacerbating the effects of O₃ exposure on roots (Grulke et al., 1998a). Increased
15 N availability also increases foliage turnover: fewer needle age classes are retained (Gower
16 et al., 1993). Therefore, the combination of both increased N and O₃ exposure increases foliar
17 turnover. Finally, N deposition and increased plant N nutrition can increase stomatal
18 conductance, leading to increased O₃ uptake. Alternatively, increased N counteracts the effect
19 of O₃ on photosynthesis by increasing photosynthetic pigments and enzymes. Nitrogen
20 deposition may mitigate the degree of foliar injury from oxidant pollution via higher available
21 N for reparation of photosynthetic pigments. Nitrogen amendments also modify the antioxidant
22 defense system in complex ways (Polle, 1998).

23 Attributes of O₃ injury to trees (foliar injury, needle retention, and canopy transparency),
24 as well as presence of pathogens and insect infestation, are routinely inventoried in established
25 sample plots distributed on Federal lands across the United States (Forest Health Protection,
26 USDA Forest Service). Foliar injury to several widespread, herbaceous species nationally
27 recognized as sensitive (bioindicators) is also assessed (U.S. National Park Service, 2003). This
28 assessment is part of a larger assessment of forest tree growth and dynamics (the Forest
29 Inventory and Analysis Program) (Smith et al., 2003; Smith, 2002)). Risk of O₃ injury is then
30 estimated for the dominant forest tree species in the sample plots. For example, 12% of sampled
31 black cherry, 15% of loblolly pine, and 24% of sweetgum (*Liquidambar styraciflua*) were found

1 to be in the highest risk category in the northeast and mid-Atlantic states (Coulston et al., 2003).
2 In the Carpathian Mountains, 12 to 13% of all trees (broadleaf and coniferous) have greater than
3 26% crown defoliation (Bytnerowicz et al., 2002b). In general, broadleaves (primarily beech)
4 trees were less affected (8 to 45%) than spruce (up to 37%) and fir (up to 50%) (Bytnerowicz
5 et al., 2002b). Ozone injury was directly correlated with cumulative O₃ exposure in the Sierra
6 Nevada Mountains (Arbaugh et al., 1998); with the best correlation being found across sites
7 where >90% of the trees had O₃ injury. Although direct links of visible foliar symptoms induced
8 by O₃ to adverse effects on biomass are not always found, visible foliar symptoms have been
9 linked to decreased vegetative growth (Karnosky et al., 1996; Peterson et al., 1987; Somers
10 et al., 1998), as well as reproductive function (Black et al., 2000; Chappelka, 2002).

11 Foliar O₃ injury has also been associated with adverse effects on competitive ability and
12 survival in forest communities (Karnosky, 1981; Karnosky et al., 2003a; McDonald et al., 2002).
13 Competition can alter organism condition and affect susceptibility to O₃. Ponderosa pine
14 seedlings were more susceptible to O₃, as determined by decreased plant biomass, when grown
15 in competition with blue wild-rye grass (Andersen et al., 2001). Similarly, the magnitude of O₃
16 effects on height and diameter growth depended on the competitive status of *Populus*
17 *tremuloides* trees (McDonald et al., 2002). These studies show the importance of including
18 competition as a concurrent stressor in assessing whole plant responses to O₃. The age of the
19 community (“time since disturbance”) may also affect the ability of individuals to effectively
20 respond to O₃. Unfortunately, the vast majority of O₃ studies have been conducted on
21 open-grown plants, often grown in pots where competition is absent both above and below
22 ground.

23 Clearly, age-dependent O₃ responsiveness and juvenile-mature correlations remain
24 important research questions in attempting to scale up to ecosystem level responses. Patterns of
25 allocation between root, stem, and leaf differ between immature and mature trees. Tree
26 architecture varies with tree age, and leaf area distribution in space and time may change in
27 response to elevated O₃. All of these factors influence gas exchange in the canopy.
28 Furthermore, there may be few generalities that can be made from seedling to mature tree
29 response to O₃ within a plant functional group (Karnosky, 2003; Norby et al., 1999).
30 Consequently, modeling ecosystem response is limited to either dealing with monospecific
31 plantations or assigning average responses to a mix of species.

Below-Ground Responses

The effect of O₃ 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 O₃ can have rapid and significant effects on root growth. In many cases, decreased allocation to roots in response to O₃ occurs quickly, with reductions in root growth occurring within one growing season (Andersen and Rygielwicz, 1995a; Andersen and Rygielwicz, 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 O₃ stress.

Decreased C acquisition leads to reduced carbohydrate levels and storage pools in O₃-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., 1976). 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 O₃ 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 O₃ could not be completely separated from other known stresses across the pollutant gradient, but it appeared that O₃ was an important factor in the patterns observed.

1 Decreased storage pools can lead to carry-over effects on root growth that are compounded
2 over time. Decreased carbohydrate storage pools were associated with decreased root growth
3 during the spring following exposure to O₃, even in the absence of additional O₃ exposure
4 (Andersen et al., 1991, 1997). Decreased spring root growth was attributed to decreased stored
5 C reserves as well as to premature loss of older foliage age classes during the previous fall.
6 Aside from the loss of photosynthetic surface area associated with premature senescence, early
7 loss of foliage in the fall occurs when allocation to roots is at a maximum in many species
8 (Kozłowski and Pallardy, 1997). Older needle age classes preferentially allocate photosynthate
9 basipetally to stems and roots (Gordon and Larson, 1970; Rangnekar et al., 1969); and the loss of
10 older needles in the fall during allocation to root growth and storage; and in the spring during
11 periods of root growth, preferentially impacts roots and root processes.

12 Ozone has also been shown to affect root metabolism as evidenced by changes in root
13 respiration. Edwards (1991) found decreased root and soil CO₂ efflux during a 2-year exposure
14 of loblolly pine to O₃. Fine root respiration increased in mature red oak exposed to O₃, while
15 total soil CO₂ efflux increased in the spring and decreased in the summer and fall (Kelting et al.,
16 1995). The authors attributed increased root respiration to increased nutrient uptake in support
17 of increased demands in the shoot. Ozone decreased root system respiration in aspen after
18 12 weeks of exposure, but the decrease was closely associated with decreased root biomass and
19 probably not metabolic processes (Coleman et al., 1996). Whether other metabolic shifts occur
20 in the roots of plants exposed to O₃ needs to be examined.

21 Measurable effects on roots may occur before effects on shoots are observed because
22 shoots have immediate access to C for repair and compensation, whereas roots must compete
23 with shoots for C. Mortensen (1998) found decreased root, but not shoot, growth in *Betula*
24 *pubescens* at O₃ exposures of 42 nMol mol⁻¹L (applied 12 h day⁻¹), whereas both root and shoot
25 growth were reduced at higher exposures. Chromosomal aberrations were found in root tips of
26 Norway spruce exposed to O₃, even in the absence of biochemical changes in needles (Wonisch
27 et al., 1998, 1999). Using relatively high O₃ concentrations (0.15 ppm O₃ 6 h day⁻¹), Hofstra
28 et al. (1981) found metabolic changes in *Phaseolus vulgaris* root tips prior to the development of
29 leaf injury. Morphological changes in root tips occurred within 2 to 3 days, and metabolism
30 declined within 4 to 5 days of initiation of O₃ exposure.

1 Feedback signals from roots can influence the degree of O₃ response. Stolzy et al. (1964)
2 exposed tomato roots to periods of anaerobic conditions and tracked a change in leaf
3 susceptibility to O₃. An exposure of roots to low oxygen conditions for 3 h did not alter
4 photosynthesis, but foliar damage was decreased when the roots were subsequently exposed
5 to O₃. In this case, a signal originating in the root appeared to alter leaf sensitivity to O₃, the
6 signal possibly being hydraulic in nature and leading to decreased O₃ uptake.

8 **SYMPTOMS OF DISEASE OR TRAUMA, SIGNS OF DISEASE**

9 Although insects and diseases are dynamic components of forest ecosystems, trees can be
10 especially susceptible to outbreaks due to the presence of multiple stressors such as drought and
11 pollutant exposure. Ozone can have direct effects on insect or disease organisms, indirect effects
12 on the insect or pathogen through changes to the host, and direct or indirect effects on natural
13 enemies of the insect or pathogen (Pronos et al., 1999). A full discussion of O₃ effects on insect
14 and pathogen interactions can be found in Section AX9.4.

15 Although the multitude of interacting factors makes it difficult to identify causative factors
16 in the field, some recent examples suggest a role for O₃ in the timing or magnitude of disease
17 attacks in the field. After periods of drought stress (such as 1995 in central Europe), the
18 incidence of bark beetle (*Ips* spp.) appears to increase. In 1998 and 1999, the mean daily capture
19 of *Ips* was lowest in plots with low O₃ exposure; the converse was also true (Grodzki et al.,
20 2004). Elevation confounded the relationships, but the differences in *Ips* frequency in relation to
21 O₃ concentrations were highly significant at lower elevations. In the Valley of Mexico, a 1982
22 to 1983 drought was documented, but not described as precipitating a bark beetle attack in the
23 early 1980s. However, a link between air pollutant exposure and bark beetle attacks were
24 implicated, because attacked trees were already O₃-stressed at the time of the bark beetle attack
25 (Alvarado et al., 1993).

26 An early study showed that oxidant exposure predisposed Ponderosa pine to the root
27 pathogen *Fomes annosus* (James et al., 1980). Both root diseases (Pronos et al., 1999) and O₃
28 exposure (Grulke and Balduman, 1999) can each reduce root biomass, leading to increased
29 drought stress, insect attack, and subsequent windthrow or death. Trees may take several years
30 to die, and the patterns of precipitation and annual total rainfall interact to drive the level of

1 drought stress experienced by the tree (Pronos et al., 1999). Additional research is necessary to
2 fully understand the complex interactions occurring between O₃ stress and other biotic stresses.
3

4 **AX9.6.6 Ecosystem, Chemical, and Physical Characteristics (water, soil)**

5 **AX9.6.6.1 Nutrient Concentrations, Trace Inorganic and Organic Chemicals**

6 Ozone exposure reduces the nutritional content of tissues, as well as causing elemental
7 imbalances. Foliar nutrient content may be too high (toxic) or too low (deficient), but the
8 relative amounts and ratios among all nutrients can also result in imbalances.

9 Although N deposition and foliar N content increased with O₃ exposure in the San
10 Bernardino Mountains, K, Mg, Fe, and Al were all also higher in Ponderosa pine at sites more
11 exposed to air pollution (Poth and Fenn, 1998). Trees with greater foliar injury (due to O₃
12 exposure) had higher current-year needle concentrations of P, K, Zn, and Fe than trees at the
13 same site that were less injured. In drought-stressed Ponderosa pine with O₃ exposure, foliar N
14 was also elevated and retained in the remaining needles (Temple et al., 1992). At a relatively
15 clean site in the eastern San Bernardino Mountains, N, P, and K were efficiently reabsorbed, but
16 the amount of P remaining in the foliage was relatively high compared to defined thresholds.
17 The fact that other elements were modified besides the N being deposited emphasizes the degree
18 of chemical imbalance in the tissue. Foliar micronutrients were within the normal ranges
19 reported for Ponderosa pine (Poth and Fenn, 1998; Powers, 1981). Because both N deposition
20 and O₃ exposure reduce root biomass, it was unlikely that the foliar nutrient content was higher
21 due to greater uptake. Instead, it appears that retranslocation from senescing tissue was
22 responsible. Across a pollution gradient in the Carpathian Mountains in eastern Europe, only
23 S/N (expected due to high S deposition) and Fe/Mn ratios were out of balance relative to
24 established norms. The S was relatively high due to SO₂ deposition, and the Fe was relatively
25 high due to smelter plumes. No imbalances could be directly attributed to O₃ exposure
26 (Mankovska et al., 2004).

27 28 **AX9.6.7 Ecological Processes**

29 **AX9.6.7.1 Energy Flow**

30 All green plants generate and use energy-containing C compounds through the processes of
31 photosynthesis and respiration. Whole-plant C uptake is dependent on photosynthesis rates, leaf

1 area, and leaf phenology. The effects of O₃ at the site of action in the leaf are discussed in
2 Section AX9.2. Here, the main focus is on whole-plant carbon dynamics resulting from changes
3 in C acquisition or use under O₃ stress.

4 In natural ecosystems, O₃ has been shown to depress photosynthesis in sensitive tree
5 species including Ponderosa pine (Grulke et al., 2002b; Miller et al., 1969; Takemoto et al.,
6 1997; Weber et al., 1993) and aspen (Coleman et al., 1995a; Noormets et al., 2001a,b; Sharma
7 et al., 2003; Yun and Laurence, 1999). In a study of mature Jeffrey pine, trees in mesic
8 microsites had greater O₃ uptake over the growing season in comparison to trees in xeric
9 microsites (Grulke et al., 2003a) and greater O₃ uptake was correlated with lower mid-canopy
10 needle retention, lower branch diameters, and lower foliar N content (Grulke et al., 2003a).
11 Chamber studies have also shown negative effects of O₃ on tree seedling canopy structure
12 (Dickson et al., 2001) and leaf area (Neufeld et al., 1995; Wiltshire et al., 1994). It is well
13 known from chamber and field studies that O₃ exposure is correlated with lower foliar retention
14 (Grulke and Lee, 1997; Karnosky et al., 1996; Miller et al., 1963, 1972; Pell et al., 1999; Topa
15 et al., 2001).

16 In contrast to the relatively consistent findings for photosynthesis, O₃ effects on respiration
17 have been more variable. Stem respiration was unaffected by O₃ exposure (Matyssek et al.,
18 2002), suggesting that construction costs of new stems are not affected by O₃. However, the bole
19 represents a relatively large storage pool of carbohydrates in mature trees, and the timing of
20 phenological events among individual trees may help to confound the ability to statistically
21 detect differences in stem respiration across pollutant gradients (Grulke et al., 2001). Below-
22 ground respiration has been found to both increase and decrease in response to O₃, depending on
23 the approach and timing of CO₂ measures (Andersen and Scagel, 1997; Coleman et al., 1996;
24 King et al., 2001; Scagel and Andersen, 1997). The decreased soil respiration is thought to be
25 due to reduced root growth under O₃ exposure, but could also be partially explained by
26 decreased microbial respiration in response to O₃. Additional research is necessary to identify
27 the role of O₃ in affecting root versus heterotrophic respiration, particularly over long time
28 intervals.

29 Carbohydrate availability and use influence the degree to which plants respond to O₃
30 exposure. A model simulation of the effect of O₃ exposure on bole growth of Ponderosa pine
31 showed a 15% reduction in mass (Weber and Grulke, 1995), largely influenced by differences in

1 carbohydrates allocated and partitioned in repair processes elsewhere in the tree. Foliar
2 respiration is thought to increase under elevated O₃ because maintenance costs (energy needs) of
3 leaves damaged by O₃ are higher than normal (Grulke and Balduman, 1999; Noormets et al.,
4 2001b). However, differences in foliar respiration are subtle and difficult to detect statistically.
5 Foliar carbohydrate studies also suggest that more C is used under O₃ stress for repair processes
6 (Grulke et al., 2001; Topa et al., 2001) which would result in increased respiration. Ozone
7 exposure also reduced enzymatic activities of carbohydrate metabolism related to the breakdown
8 of sucrose (Einig et al., 1997). Changes in soil respiration in response to O₃, even though O₃
9 does not penetrate into the soil, illustrates the tight coupling of plant C balance and soil biota and
10 illustrates the potential role O₃ plays in altering ecosystem C balances (Andersen, 2000).

11 Ozone can affect plant allometry through changes in energy use, potentially affecting net
12 primary production (NPP) at larger scales. The net effect of O₃ impacts on photosynthesis and
13 respiration for sensitive components of natural ecosystems is that height growth (Isebrands et al.,
14 2001; Oksanen, 2003a) and radial growth (Isebrands et al., 2001; Oksanen, 2003b; Peterson
15 et al., 1987, 1991) can be negatively affected by O₃. This has been extrapolated to decreased
16 NPP (Hogsett et al., 1997; Laurence et al., 2000).

17 Energy flow in plant communities can be altered by O₃ through changes in C allocation.
18 It is well known that elevated O₃ affects C allocation to roots (Andersen et al., 1997; Coleman
19 et al., 1995b; Grulke et al., 1998a, 2001; Grulke and Balduman, 1999) by decreasing or
20 inhibiting phloem loading of carbohydrates (Grantz and Farrar, 1999; Landolt et al., 1997), or of
21 carbohydrate metabolism (Einig et al., 1997). This leads to depressed root growth (Andersen
22 et al., 1991; Coleman et al., 1996; Grulke et al., 1998a) and the potential for plant communities
23 to have an increased susceptibility to drought through altered root-shoot balance. Furthermore, it
24 can negatively affect below-ground food webs (Phillips et al., 2002; Scagel and Andersen,
25 1997).

26 Another energetically costly response to O₃ exposure is that the production of defense
27 compounds, such as antioxidants, tend to increase under elevated O₃ conditions (Sheng et al.,
28 1997; Tausz et al., 1999c, 2002). Antioxidants help the plant scavenge free radicals before they
29 can cause damage to membranes or cell walls, but they demand C for production such that
30 growth can be adversely affected. In mature Jeffrey pine, stomatal uptake of O₃ elicited one
31 complex of antioxidant defenses in mesic microsites, while endogenously generated free radicals

1 in the chloroplast elicited a second complex of antioxidant defenses in xeric microsites (Grulke
2 et al., 2003b).

4 **AX9.6.7.2 Material Flow**

5 Plants as producers are responsible for using inorganic atmospheric C and reducing it into
6 organic forms used by consumers, thus driving nutrient processes in ecosystems. Ozone has the
7 potential to disrupt material flow through organic C cycling and changes in nutrient cycling,
8 particularly N and P cycling. Although there is indirect evidence that O₃ is disrupting C and
9 nutrient cycling at the ecosystem level, there is little direct evidence that O₃ alters nutrient
10 processing at ecosystem scales.

11 The greatest annual nutrient and C input to ecosystems is from foliar and root turnover.
12 Excision of plant parts and whole plant mortality are potentially much larger, but syncopated,
13 ecosystem inputs. Ozone exposure alters C cycling in the ecosystem by affecting the
14 within-plant C allocation and partitioning in dominant, O₃-sensitive plants, and through chemical
15 composition and rate of decomposition of sloughed plant parts (roots, branches, leaves)
16 (Figure AX9-19).

17 In addition to O₃-induced changes in the quantity of C and nutrient inputs into ecosystems,
18 O₃ also can alter the nutrient quality of inputs. Ozone exposure alters nutrient levels in the
19 foliage (Boerner and Rebbeck, 1995; Fenn and Poth, 1998; Lindroth et al., 2001; Momen et al.,
20 2002) and affects the C:N ratio (Andersen et al., 2001; Grulke et al., 2003b; Grulke and Lee,
21 1997; Lindroth et al., 2001). Concentrations of compounds such as tannins, lignin, and
22 phenolics (Baumgarten et al., 2000; Findlay et al., 1996; Kim et al., 1998; Saleem et al., 2001)
23 are also affected by O₃ exposure, which in turn alters decomposability (Fenn and Dunn, 1989)
24 and litter buildup in the ecosystem.

25 There are several possible pathways by which O₃ may affect litter quality and, hence, litter
26 decomposition, thus altering nutrient flow in ecosystems. These include altered C quality,
27 altered nutrient quality, and alteration of leaf surface organisms important in decomposition
28 pathways. For example, yellow poplar and black cherry litter exposed to O₃ showed greater N
29 loss during decomposition than charcoal-filtered controls, although mass loss did not vary
30 among O₃ treatments (Boerner and Rebbeck, 1995). Subsequent studies showed that although
31 foliar N was not affected by O₃ exposure in yellow poplar leaves, foliage decomposed more

1 slowly (Scherzer et al., 1998). Other studies have also shown a change in foliar N concentration
2 in response to O₃ treatment, affecting the C:N ratio and possible litter quality (Andersen et al.,
3 2001).

4 In some cases, it appears that N remobilization from foliage into the plant is not complete
5 at the time of foliage abscission in O₃-exposed plants (Findlay and Jones, 1990; Matyssek et al.,
6 1993; Patterson and Rundel, 1995; Stow et al., 1992). Greater N content of senesced litter could
7 increase rates of decomposition. When O₃-exposed cottonwood (*Populus deltoides*) leaves
8 abscised at the same time as control leaves, they decomposed at similar rates; however,
9 prematurely senesced foliage from O₃-exposed cottonwood decomposed more slowly than
10 controls despite their higher N content (Findlay et al., 1991; Findlay and Jones, 1990). Higher N
11 in senesced leaves appeared to be related to organic complexes formed by bound phenolics
12 in O₃-exposed leaves, making the litter less palatable to decomposers, and slowing
13 decomposition rates (Findlay et al., 1996; Jones et al., 1994). Increased phenolics also have
14 been found in European silver birch (*Betula pendula*) exposed to O₃ (Saleem et al., 2001).

15 Carbon quality in leaf litter also changes in O₃-exposed foliage. Compositional changes in
16 leaf structural characteristics, such as lignin content, would be expected to alter rates of litter
17 decomposition (Fogel and Cromack, 1977; Kim et al., 1998; Meentemeyer, 1978). Blackberry
18 litter exposed to elevated O₃ had greater permanganate lignin than the control treatment, a
19 difference that was inversely related to mass-loss rates in decomposition studies (Kim et al.,
20 1998).

21 Ozone may affect early stages of decomposition by altering populations of leaf surface
22 organisms before or after senescence. Magan et al. (1995) found a shift in phyllosphere fungi on
23 Scots pine, Sitka spruce (*Picea sitchensis*), and Norway spruce exposed to O₃, but the potential
24 effect of these changes on subsequent litter decomposition was uncertain. The slowest
25 decomposition rates of preexposed blackberry leaves were found when senesced foliage was
26 exposed to O₃ during decomposition, suggesting a possible direct effect of O₃ on microorganisms
27 in decomposing litter (Kim et al., 1998). Whether O₃ concentrations at the soil surface influence
28 initial stages of litter decomposition remains to be addressed.

29 Ozone exposure also reduces nutritional content of foliage because of the degradation of
30 chlorophyll. Reconstruction of chlorophyll may be limited by nutritionally poor soils or low soil
31 moisture, as well as alteration of root uptake by O₃ exposure and other stressors. Foliar exposure

1 to O₃ may also increase leaching of nutrients (Kerstiens and Lendzian, 1989). Ozone exposure
2 promotes early senescence of foliage (Heath and Taylor, 1997; Miller and Elderman, 1977), with
3 higher nutrient content than if excised later in the growing season (Poth and Fenn, 1998).

4 Since O₃ can slow decomposition through changes in leaf quality and quantity, leaf litter
5 can accumulate (Fenn and Dunn, 1989). The accumulation of soil organic matter from increased
6 leaf litter, even in the absence of acidic deposition, can lower soil pH (Binkley, 1992). Lower
7 soil pH can promote loss of nutrients, particularly cations, from the system, further reducing
8 nutrient availability to the plant. Complex organic compounds in decomposing litter may also
9 tie up nutrients, rendering them less available to plants.

10 Other factors such as nutrient deposition also affect the degree to which O₃ influences C
11 and nutrient flow through ecosystems. In high-pollution sites, the effect of N deposition is
12 difficult to separate from O₃ exposure, and reviews of the effect of acidic (and N) deposition on
13 ecosystem nutrient dynamics are important to consider (see Binkley [1992]; Fenn et al. [1998,
14 2003b]). For example, at moderately high pollution sites, foliar content of N is higher than that
15 at lower pollution sites, but so are P, Mg, and Fe contents (Poth and Fenn, 1998). Although
16 significant changes in foliar tissue chemistry have occurred in response to long-term pollutant
17 deposition in the Carpathians (Fenn et al., 2002; Mankovska et al., 2004), much of this response
18 is correlated to heavy metal and N and S deposition. At this point, the contribution of O₃
19 exposure alone cannot be isolated without careful between-site comparisons of plant response
20 and understanding the depositional velocities of constituent atmospheric species. The significant
21 effects of plant response along known pollution gradients are important to consider, because
22 heavy metal and N and S deposition has significantly declined over the last decade, while O₃
23 exposures remain high and declines in forest health have been sustained.

24 Models provide a means to track material flows through ecosystems. Two biogeochemical
25 models were parameterized to capture long-term effects of O₃ exposure, N deposition, and
26 climate on a Ponderosa pine-dominated site in the eastern San Bernardino Mountains.
27 Simulated O₃ exposure resulted in faster production and turnover of foliage and a shift in C from
28 the canopy (15% reduction) to the forest floor (increase of 50 to 60%) (Arbaugh et al., 1999).
29 When O₃ exposure was combined with that of N deposition, litter mass increased exponentially.

30 The direct effect of O₃ exposure on below-ground nutrient dynamics and ecosystem
31 material flow is poorly understood. Additional research will be necessary to understand spatial

1 and temporal dynamics of nutrient and C flow in ecosystems and to separate the effects of O₃
2 from those attributable to N deposition.

4 **AX9.6.8 Hydrological and Geomorphological**

5 At present, there are no publications on the effects of O₃ exposure that are carried through
6 at the ecosystem level to changes in mass water flow, channel morphology, riparian habitat
7 complexity, or sediment movement. It is possible that processes occurring at smaller scales are
8 affecting geomorphological processes in ecosystems; however, difficulty in scaling these
9 responses spatially and temporally have made it difficult to show experimentally. It is possible
10 that O₃ exposure affects water quality through changes in energy and material flows, as
11 discussed previously.

13 **AX9.6.9 Natural Disturbance Regimes**

14 There has been little research on how natural disturbances interact with O₃ to affect
15 performance of plants, communities, and ecosystems. The frequency, intensity, extent, and
16 duration of natural disturbances are variable and unpredictable. However, there have been
17 enough ecophysiological studies to suggest that O₃ could predispose plant communities to
18 certain natural stresses, e.g., drought stress or extreme low-temperature stress during the winter.

19 While several studies have shown that drought stress reduces O₃ uptake through stomatal
20 closure, evidence also suggests that O₃ can alter plant water use and susceptibility to drought. In
21 controlled studies, Reich and Lassoie (1984) showed that relatively low O₃ concentrations could
22 diminish stomatal control and alter water use efficiency. Ash trees (*Fraxinus excelsior*) exposed
23 to elevated O₃ had greater water use early in the growing season, but less water use late in the
24 growing season when exposed to elevated O₃ (Wiltshire et al., 1994). Under moderate drought
25 stress, Norway spruce trees grown under elevated O₃ consumed water faster and showed higher
26 stomatal conductances than controls (Karlsson et al., 1995). Pearson and Mansfield (1993)
27 showed that successive O₃ episodes disrupted stomatal function, making beech seedlings more
28 susceptible to drought. Previous year O₃ exposure was shown to have a carry-over effect in the
29 following growing season for beech (Pearson and Mansfield, 1994).

30 Few studies showing the effects of O₃ on water relations of field-grown trees are found in
31 the literature. However, Grulke et al. (2003a) examined the effects of O₃ on canopy transpiration

1 of Jeffrey pine from mesic and xeric microsites and found that trees from mesic sites had 20%
2 more O₃ uptake than those in the xeric sites. The authors also concluded that the mesic trees had
3 greater O₃ injury as evidenced by lower needle retention, whereas trees in xeric microsites had
4 greater chlorotic mottle. Chlorotic mottle induced by stomatal uptake of O₃ is indistinguishable
5 from that of endogenously produced oxidants resulting from partially closed stomata, a reduction
6 of CO₂ inside the leaf, and production of strong oxidizers within the chloroplast when excited
7 electrons are passed to O₂ instead of CO₂ under high light levels.

8 Trees living near the limits of their freezing-tolerance range may be especially susceptible
9 to predisposition of freezing injury by O₃ (Sheppard et al., 1989). However, Aleppo pine
10 exposed to elevated O₃ had enhanced winter hardiness (Wellburn and Wellburn, 1994). As with
11 the seasonal carryover of drought susceptibility, the influence of elevated O₃ on freezing
12 tolerance is carried over from summer to winter. Such effects have been demonstrated for Sitka
13 spruce (Lucas et al., 1988) and for red spruce (Waite et al., 1994). Sorting out the role of
14 elevated O₃ in contributing to frost or low-temperature damage in forests remains difficult due to
15 the presence of other factors that may affect senescence.

17 **AX9.6.10 Scaling to Ecosystem Levels**

18 The vast majority of literature describing O₃ effects comes from short-duration herbaceous
19 plant or tree seedling studies under controlled conditions. Scaling results from these studies
20 requires extrapolation over both space and time in order to understand the full extent of changes
21 in ecosystems. In addition to spatial, temporal, and age-related complexities, ecosystems are
22 composed of organisms whose lifetimes range from hours to centuries (Laurence and Andersen,
23 2003). Forested ecosystems are affected by environmental conditions such as water and nutrient
24 availability, as well as by intra- and interspecies competition. Therefore, direct experimentation
25 to determine the response of forested ecosystems is not simply a matter of determining the effect
26 of O₃ on individual mature trees. In addition, even if an experiment can be conducted,
27 extrapolation of the results across landscapes and regions remains challenging. Nonetheless,
28 models provide a means to explore possible long-term changes and to identify important
29 research uncertainties.

30 Approaches to scaling fall roughly into two categories: (1) process-based modeling to
31 extrapolate physiological responses to O₃ based on seedling studies and (2) field assessments

1 using surveys and growth correlations, often in association with stand-level models to address
2 ecosystem complexity. Comparatively good information is available on process level effects
3 of O₃ in seedlings and, therefore, some models offer the opportunity to use this information to
4 scale O₃ effects at the stand and regional scales (Chappelka and Samuelson, 1998; Fuhrer et al.,
5 1997; Hogsett et al., 1997; Laurence et al., 2000). Field assessments offer the opportunity to
6 examine larger plot sizes, older trees, and trees growing under realistic competition. These two
7 approaches are discussed in more detail below.

9 **AX9.6.10.1 Scaling from Seedlings to Mature Trees**

10 A number of investigators have used simulation models based on physiological processes
11 to integrate available data and predict the effects of O₃ on mature trees. Such models predict tree
12 growth by simulating fundamental mechanisms, rather than through a statistical analysis of
13 empirical data. For instance, the process of photosynthesis is simulated based on environmental
14 conditions and physiological characteristics, and then the fixed C is allocated to plant growth
15 using principles of plant physiology. Models based on mechanisms should be applicable across
16 wide areas if the important functional relationships are represented accurately in the models and
17 if the environmental conditions are accurately identified. The ability of six models (TREGRO,
18 CARBON, ECOPHYS, PGSM, TREE-BGC, and W91) to simulate the effects of climatic
19 change and O₃ have been reviewed by Constable and Friend (2000). Of these models, only
20 PGSM and TREGRO explicitly simulated the effects of O₃ on foliar processes.

21 The TREGRO model was used to simulate C allocation and tissue growth in seedlings and
22 mature red oak trees based on the experimental data discussed above (Weinstein et al., 1998).
23 For seedlings at 2×-ambient O₃, only the total nonstructural carbohydrate (TNC) storage pool
24 was predicted to be affected. For mature trees, large decreases were predicted for TNC, leaves,
25 stem, branch, and both fine and coarse roots. Most predicted effects in mature trees were
26 consistent with observations in the field, but the simulations overestimated the effect of
27 2×-ambient O₃ on root TNC and growth. The authors suggested that this discrepancy may have
28 been due to trees reducing respiration in response to O₃ stress, a response not simulated in the
29 model.

30 For *Abies concolor*, TREGRO was parameterized and simulated growth of a mature tree
31 for 3 years to test for effects of O₃ exposure and drought stress (Retzlaff et al., 2000).

1 Reductions in O₃ exposure-mediated carbon assimilation were translated to losses in whole tree
2 biomass that probably would not be detectable in the field. However, TNC levels in branch
3 tissue were simulated to be lowered by over 50%, and branch growth was reduced in a
4 moderately polluted site relative to a clean site. Low O₃ exposure (sufficient to decrease C
5 assimilation by 2.5%) and drought stress (25% reduction in annual precipitation, which is
6 common on a decadal scale) acted synergistically to reduce C gain of whole tree biomass of
7 *A. concolor*. Simulated results of the tests were comparable to effects found in OTCs for
8 seedlings and pole-sized trees in clean and moderately polluted sites.

9 Models such as TREGRO are usually parameterized from many different sources of data,
10 including chamber experiments and plantations, from seedlings to mature trees, making it
11 difficult to validate that they reproduce changes that occur as trees develop from seedlings to
12 maturity. To address this issue, physiological and growth data were collected from a natural
13 stand of *P. ponderosa* and used to parameterize the TREGRO model (Gulke and Retzlaff,
14 2001). Representative trees of each of five tree age classes were selected based on population
15 means of morphological, physiological, and nearest neighbor attributes. Seedlings were
16 observed to differ significantly from pole-sized and older trees in most physiological traits. The
17 changes in biomass with tree age predicted from the model closely matched those of trees in the
18 natural stand.

19 The PGSM model was used to simulate *Pinus ponderosa* seedling growth responses to O₃
20 exposure and drought stress (Chen et al., 1994). Drought stress was predicted to reduce the
21 effect of O₃ on growth, as was observed in the experimental data. The TREGRO model was
22 used to simulate responses of Pacific Coast and interior varieties of *P. ponderosa* to five
23 simulated O₃ exposures between subambient and 3×-ambient (Constable and Taylor, 1997).
24 Simulated growth of var. *ponderosa* was reduced more than that in var. *scopulorum* with all O₃
25 exposures. Drought was protective of O₃ exposure. Similar results were also found in a
26 relatively moist Ponderosa pine plantation (Panek and Goldstein, 2001), whereas drought was
27 synergistically deleterious with cumulative O₃ exposure in a natural stand (Gulke et al., 2002b).

28 For *Pinus taeda*, the Plant Growth Stress Model (PGSM) was calibrated with seedling data
29 and then used to simulate the growth of mature trees over a 55 year period in the Duke Forest,
30 NC, using estimates of historical O₃ concentrations (Chen et al., 1998). Simulated stem diameter
31 and tree height were comparable to observed values. In another simulation using TREGRO,

1 loblolly pine was more sensitive (greatest reduction in C gain) to a peak O₃ episode in July
2 (Constable and Retzlaff, 1997), whereas mature yellow poplar (*Liriodendron tulipifera*) was
3 more sensitive to a peak O₃ episode in August.

4 For *Populus tremuloides*, the ECOPHYS model was used to simulate the relative above-
5 ground growth response of an O₃-sensitive clone (259) exposed to square-wave variation in O₃
6 concentration (Martin et al., 2001). The model adequately simulated several effects of O₃,
7 including a greater effect on stem diameter than on stem height, earlier leaf abscission, and
8 reduced stem and leaf dry matter production at the end of the growing season. For *Acer*
9 *saccharum*, the TREGRO model was used to predict effects of a 10-year O₃ exposure on root and
10 stem growth of a simulated 160-year-old tree (Retzlaff et al., 1996). Twice-ambient O₃ exposure
11 (for Ithaca, NY) was predicted to deplete the TNC pools and reduce fine root production.
12

13 **AX9.6.10.2 Surveys, Growth Correlations and Stand-Level Modeling**

14 Stand-level studies have included surveys of O₃ symptoms, correlations of radial growth
15 with O₃ and other environmental factors, and regional-scale modeling. In addition, open air O₃
16 exposure systems, such as those being used on *Populus tremuloides*-mixed stands in northern
17 Wisconsin (Karnosky et al., 2003b) and on *Fagus sylvatica* and *Picea abies* in Germany (Nunn
18 et al., 2002) offer an opportunity to examine larger plot sizes, older trees, and trees growing
19 under realistic competition. Plots along natural O₃ gradients, as have been used very effectively
20 in southern California forest studies (Miller and McBride, 1999b) and with *P. tremuloides* stands
21 in the Great Lakes region (Karnosky et al., 1999), offer additional insights into ecosystem level
22 responses. Undoubtedly, however, simulation modeling will have to become an integral
23 component of research in order to predict adequately ecosystem responses to O₃ (Laurence and
24 Andersen, 2003). Results of these approaches are discussed below, organized into three U.S.
25 regions: (1) northern states (including the upper Midwest and the Northeast), (2) southeastern
26 states, and (3) western states (primarily California). A fourth section contains selected
27 information from Europe.
28

29 ***Northern and Midwestern United States.*** In recent years, the USDA Forest Service has
30 conducted systematic O₃ biomonitoring surveys in most north-central and northeastern states
31 (Coulston et al., 2003; Smith et al., 2003). Plots are located on a systematic grid, and trained

1 field crews evaluate up to 30 plants of up to six species that have foliar injury symptoms
2 diagnostic of O₃ damage. For the United States as a whole, injury has been found more often in
3 eastern than in interior or west-coast states. As expected, O₃ injury is more common and more
4 severe in areas with higher O₃ concentrations. Of sampled *Prunus serotina* plots, ~12% were
5 estimated to be at high risk for injury based on a injury index derived from the survey data
6 (Coulston et al., 2003). *P. serotina* was estimated to be at risk for injury on the Allegheny
7 Plateau and the Allegheny Mountains (in Pennsylvania, West Virginia, and Maryland), as well as
8 in the coastal plain of Maryland and Virginia.

9 Ozone concentration, foliar injury, and physiological traits were measured on *P. serotina*
10 trees of different sizes in Pennsylvania (Fredericksen et al., 1995). The proportion of foliage
11 injured was 46% for seedlings, 15% for saplings, and 20% for canopy trees. Cumulative O₃ flux
12 was the most useful O₃ metric for predicting injury. Injury was negatively correlated ($r^2 = 0.82$)
13 with net photosynthetic rates, but was not related to stomatal conductance. *P. serotina* is
14 discussed further below, because detailed surveys have been conducted in the Shenandoah and
15 Great Smoky Mountains NPs in the southeastern United States.

16 Over the past several decades, some surveys of white pine (*Pinus strobus*) have reported
17 significant associations between foliar injury and reduced growth (Anderson et al., 1988; Benoit
18 et al., 1982). However, a review of 93 surveys conducted from 1900 through the late 1980s
19 concluded that methodological problems were pervasive, including such issues as proximity to
20 roads, lack of peer review, lack of random sampling, small sample sizes, and lack of quantitative
21 methods to estimate severity (Bennett et al., 1994). Because of these problems, along with
22 evidence of adequate growth rates for *P. strobus* regionally and contradictory evidence from
23 numerous studies of symptom production in response to controlled O₃ exposure, these authors
24 concluded that there was no clear evidence of decline in *P. strobus* (Bennett et al., 1994).
25 A more recent study in Acadia National Park (ANP) in Maine found no association between O₃
26 exposure in OTCs and symptom development in *P. strobus*, calling into question whether
27 symptoms previously ascribed to O₃ may be caused by some other stress (Kohut et al., 2000).
28 However, another ANP study found significant correlations between O₃ exposure and the radial
29 growth of trees during 10 years in 7 of 8 stands examined (102 trees total; (Bartholomay et al.,
30 1997). Taken together, these results suggest that there may not an association between growth of

1 *P. strobus* trees and putative O₃ symptoms, but there may be an association between O₃ exposure
2 and radial growth of mature trees in the field.

3 The study of O₃ effects was undertaken from 1990 to 1993 in the ANP in Maine, because
4 this location experiences elevated O₃ exposures due to transport from urban areas located upwind
5 (Kohut et al., 2000). Thirty-two species of plants found in the park were propagated and
6 exposed to O₃ in OTCs. In addition, ambient O₃ concentrations were monitored at the study site
7 at 15 m above sea level and near the top of Cadillac Mountain at 470 m above sea level. At the
8 study site, the maximum 1-h O₃ concentration was 140 ppb, which occurred in both 1990 and
9 1991. Daytime 12-h O₃ concentrations were 35, 41, 36, and 37 ppb during the four years; and O₃
10 concentrations were consistently higher at the high-elevation site. Species showing foliar injury
11 at ambient O₃ concentrations included *Prunus serotina*, *Populus tremuloides*, *Fraxinus*
12 *americana*, *Pinus banksiana*, big-leaf aster, and spreading dogbane. Species showing foliar
13 injury at 1.5 × ambient O₃ concentrations included *Betula populifolia*, small sundrops, and
14 bunchberry. Species remaining uninjured at 2 × ambient O₃ concentrations included *Betula*
15 *papyrifera*, *Pinus strobus*, *Pinus rigida*, *Picea rubens*, *Thuja occidentalis*, *Quercus robur*,
16 Canada bluejoint grass, wild radish, and Canada mayflower. Because of their O₃ sensitivity and
17 diagnostic symptoms, big-leaf aster, spreading dogbane, *Populus tremuloides*, *Fraxinus*
18 *americana*, and *Prunus serotina* were recommended as bioindicators for the ANP.

19 The PnET-II model was applied to 64 locations across the northeastern United States to
20 simulate the effects of ambient O₃ on mature hardwood forests (Ollinger et al., 1997). In this
21 model, O₃ effects on each of several layers of the forest canopy were represented by a single
22 linear equation relating predicted O₃ uptake to decreased net photosynthetic rate. Wood growth
23 was predicted to decrease between 3 to 22%, with greatest reductions in southern portions of the
24 region where O₃ levels were highest and on soils with high water-holding capacity where
25 drought stress was absent. Little variation was predicted among years, because high O₃ often
26 coincided with hot, dry weather conditions that reduced predicted stomatal conductance and O₃
27 uptake.

28 In order to estimate the impact of O₃ on forests, effects must be evaluated not only on
29 individuals, but also on mixtures of species and the composition of forest stands. The PnET
30 model described above evaluated the effects of O₃ on broad forest types (an evergreen/deciduous
31 mix), but did not address specific forest species composition. In order to address competition

1 among species, the TREGRO model was linked to the ZELIG forest stand growth model and a
2 geographic information system was created to predict the effects of O₃ across the north-central
3 and northeastern United States (Laurence et al., 2000). ZELIG is a gap-succession model used
4 to simulate succession in mixed stands typical of eastern and northern forests. Ambient O₃
5 generally caused a reduction of 2 to 4% in the growth of *Quercus rubra* across the region during
6 the 100-year simulation. The response followed the pattern of O₃ exposure, with little effect in
7 the northwest part of the region, but with greater effect in southern locations. The O₃ response of
8 *Acer saccharum* to O₃ varied widely, but the overall growth response was always positive,
9 indicating that the evergreen/deciduous mix was able to take advantage of the decrease in the
10 growth of *Q. robur* and other species caused by O₃. In the northernmost part of the region, *A.*
11 *saccharum* growth increased by up to 3%, but in the southern part of the region, its growth
12 increased by up to 12%. The authors ascribed this enhanced growth to a combination of warmer
13 temperatures and reduction in the growth of *Prunus serotina*, a minor component of the
14 simulated stand that was very sensitive to O₃.

15
16 ***Southeast United States.*** In a survey of the Great Smoky Mountains NP, foliar injury
17 attributed to O₃ was found on 47% of the more than 1,600 plants examined (Chappelka et al.,
18 1997). In subsequent surveys of injury in the park, injury was found on mature trees of the
19 following species: *Sassafras albidum*, *Prunus serotina*, and *Liriodendron tulipifera* (Chappelka
20 et al., 1999b,c). In a similar study in Shenandoah National Park, injury was found on *Fraxinus*
21 *americana*, *P. serotina*, and *L. tulipifera* (Hildebrand et al., 1996).

22 For *Prunus serotina* seedlings grown in soil in OTCs and exposed to relatively low
23 ambient levels of O₃ in Pennsylvania, there was no correspondence between visible foliar stipple,
24 leaf gas exchange, and seedling growth between two families previously shown to differ in O₃
25 symptoms (Kouterick et al., 2000). However, significant exposure-response relationships were
26 found for foliar injury in the Great Smoky Mountains and Shenandoah National Parks. In each
27 park, foliar injury was evaluated on mature *P. serotina* trees on three plots at different elevations
28 near O₃ monitors during 1991 to 1993 (Chappelka et al., 1999a,b; Hildebrand et al., 1996). In
29 1991, incidence was 60% and 45% for the two parks and 33% in both parks during 1992 and
30 1993. Symptoms were greater at the highest elevations where O₃ concentrations were highest.
31 In another study, radial growth rates were measured in 44 *P. serotina* trees ranging in age from

1 19 to 56 years old with and without O₃ symptoms, at three sites in the Great Smoky Mountains
2 National Park. Trees with O₃ symptoms were compared to similar-sized trees with few
3 symptoms. There was no evidence that trees with O₃ symptoms had lower growth rates (p = 0.6)
4 (Somers et al., 1998).

5 In the Great Smoky Mountains National Park, radial growth rates were measured for 44 *L.*
6 *tulipifera* trees ranging in age from 30 to 58 years old, with and without O₃ symptoms, at three
7 sites at different elevations (Somers et al., 1998). Trees with O₃ symptoms averaged 30% lower
8 growth rates over ten years (p = 0.0005). Seedlings of *Liriodendron tulipifera* were exposed for
9 two seasons to 2×-ambient O₃ exposures in OTCs in Delaware, OH (seasonal SUM00 exposures
10 of 107 and 197 ppmh) (Rebbeck, 1996). Foliar O₃ symptoms were observed, but growth was not
11 reduced.

12 In order to evaluate the influence of interspecies competition on O₃ effects, the linked
13 TREGRO and ZELIG modeling system was used to predict the effects of O₃ over 100 years on
14 the basal area of species in a *Liriodendron tulipifera*-dominated forest in the Great Smoky
15 Mountains NP (Weinstein et al., 2001). Ambient O₃ was predicted to reduce the basal area of *L.*
16 *tulipifera* by 10%, whereas a 1.5×-ambient exposure was predicted to cause a 30% reduction.
17 Basal area of *Acer rubrum* and *Prunus serotina* was predicted to increase for some years, but
18 then decrease by up to 30%, with few changes in the total basal area of all species by the end of
19 the simulation.

20 In order to evaluate the influence of interspecies competition on O₃ effects, the linked
21 TREGRO and ZELIG modeling system was used to predict the effects of O₃ on the basal area of
22 *Pinus taeda* and *Liriodendron tulipifera* growth throughout their ranges (Laurence et al., 2003).
23 The models were parameterized using biological and meteorological data from three sites in the
24 southeastern United States (in Alabama, Louisiana, and North Carolina). Forest stand response
25 to five O₃-exposure regimes with annual SUM06 values ranging from 0 to 100 ppmh per year
26 was simulated for 100 years. The simulated basal area of the two species was generated within
27 the context of four other tree species common in southeastern forests. Basal area of *P. taeda* was
28 highly responsive to precipitation and O₃ exposure, with the greatest increases under high-
29 precipitation, lowO₃-exposure scenarios and the greatest decreases under low-precipitation, high
30 O₃-exposure scenarios. The basal area of *L. tulipifera* did not significantly differ (+10%) from
31 simulations using a “base case” (ambient O₃, average precipitation).

1 Systematic biomonitoring surveys found that approximately 24% of sampled sweetgum
2 and 15% of sampled *Pinus taeda* plots were estimated to be at high risk for foliar injury on the
3 coastal plain of Maryland and Virginia (Coulston et al., 2003; Smith et al., 2003). In a study in
4 Tennessee, the effect of ambient (uncontrolled) O₃ on 28 mature canopy-dominant 50 to
5 90-year-old *P. taeda* trees in five stands was measured over a 5-year period (McLaughlin and
6 Downing, 1995, 1996). Of many O₃ metrics, a 3-day average of hourly O₃ values ≥ 40 ppb
7 (AOT40) was found to best explain short-term variation in stem expansion as measured with
8 dendrometer bands. Interactions between O₃, temperature, and drought stress (as indicated by
9 the weekly moisture stress index) accounted for 63% of the short-term variation in stem growth
10 rates. Because there are interactions among O₃, drought stress, and temperature that may differ
11 with the averaging time (days to years), this type of study cannot provide conclusive proof of
12 cause and effect (Reams et al., 1995). However, the results do suggest that the effects of O₃
13 measured on loblolly seedlings may also be occurring in mature trees in both wet and dry sites.
14 The magnitude of effects of O₃ on growth, including interactions with other variables in this
15 study, ranged from 0 to 15% over 5 years, with an average of 5.5%.

16
17 **Western United States.** The USDA Forest Service conducted O₃ biomonitoring surveys in
18 Washington, Oregon, and California during one year (1998), and in the Sierra Nevada and
19 Sequoia NFs every other year for several decades (Campbell et al., 2000). Overall, only one plot
20 showed any symptoms of O₃ injury outside of the Sierra Nevada and Sequoia National Forests.
21 In the Sierra Nevada National Forest, between 30 and 40% of trees showed injury from 1989
22 through 1997. In the Sequoia National Forest, between 40 and 50% of the trees surveyed
23 showed injury from 1990 through 1998.

24 For *Pinus ponderosa* along a well-studied gradient of O₃ exposure in the San Bernardino
25 mountains, chlorotic mottle was highest on foliage at the most polluted site, as has been found
26 previously (Grulke and Balduman, 1999). Based on whole-tree harvests, root biomass was
27 lowest at the most polluted sites, confirming previous studies with seedlings under controlled
28 conditions, as discussed above. Ozone responses in highly polluted environments such as
29 Southern California may not be predicted adequately by extrapolating effects from single-factor
30 experiments. Instead, combined approaches utilizing field experiments and modeling efforts
31 may be required to properly account for a combination of stressors including O₃, N deposition,

1 and drought. Furthermore, the available studies underscore the lack of correlation between O₃
2 symptoms and mature tree effects. If a field survey fails to find a correlation between mature
3 tree growth and O₃, this result may be due to the dominant effect of another factor such as N
4 deposition and may not be evidence that O₃ does not reduce the growth of mature trees.

5 The TREGRO model was used to evaluate how projected future temperature and CO₂
6 concentrations might affect the response of individual Ponderosa pine to O₃ at seven sites in
7 California, Oregon, and Washington (Tingey et al., 2001). As expected, growth decreased with
8 increasing O₃ exposure. Differences in O₃ response among sites appeared to be due primarily to
9 differences in precipitation.

10 Often air quality standards do not translate directly into measurable improvements in tree
11 growth or productivity. To evaluate whether past improvements in air quality have improved
12 Ponderosa pine growth, TREGRO was used to simulate growth at sites in the San Bernardino
13 Mountains in California (Tingey et al., 2004). Ozone and meteorological data from the past
14 37 years was used to run the simulations. Despite variation in precipitation and temperature, O₃
15 was found to reduce simulated tree growth. The authors were able to simulate growth
16 improvements as air quality improved during the 1980s and 1990s, suggesting that
17 improvements in emission control strategies benefitted Ponderosa pine. The model simulations
18 were qualitatively consistent with improvements in canopy condition that were observed at sites
19 where O₃ reductions were the greatest.

20
21 ***Studies in Europe.*** In a 4-year study of *Fagus sylvatica* in Switzerland at 57 forest sites
22 ranging in age from 65 to 173 years, stem increment was found to decrease with increasing
23 maximum O₃ exposure (Braun et al., 1999). In this study, O₃ concentration was estimated by
24 interpolation among monitoring stations, and other site conditions such as soil water status and
25 temperature were interpolated from weather stations. Other factors such as N deposition, tree
26 diameter, and canopy dominance were also found to be significantly associated with stem
27 increment. The maximum annual O₃ dose (expressed as AOT40) was found to be more strongly
28 associated with decreased stem increment than was the average O₃ dose over the 4 years.
29 A growth reduction of 22.5% (confidence interval 14.3 to 28.6%) was associated with each
30 10 ppmh increment of O₃ (expressed as AOT40). This decrease was steeper than the 6.1%
31 growth reduction summarized previously from several OTC studies with *F. sylvatica* seedlings

1 (Fuhrer et al., 1997). However, the authors suggest that this difference may be explained largely
2 by the 4 years of exposure in their forest survey study as compared to the 1-year exposures for
3 seedlings. As with any forest survey, these results must be interpreted with caution because O₃
4 exposure was correlated with other variables, such as tree age and the deposition of NO₂
5 and SO₂.

7 **AX9.6.11 Summary of Ecological Effects of Ozone Exposure on Natural** 8 **Ecosystems**

9 In this chapter, an effort has been made to discuss the adverse effects of O₃ on natural
10 ecosystems within the context of the SAB framework for assessing and reporting ecological
11 conditions (Young and Sanzone, 2002) (Figure AX9-21). Using this framework, there is
12 evidence that tropospheric O₃ is an important stressor of natural ecosystems, with
13 well-documented impacts on the biotic condition, ecological processes, and chemical/physical
14 nature of natural ecosystems. In turn, the effects of O₃ on individual plants and processes are
15 scaled up through the ecosystem affecting processes such as energy and material flow, intra- and
16 interspecies competition, and NPP. Thus, effects on individual keystone species and their
17 associated microflora and fauna may cascade through the ecosystem to the landscape level. This
18 suggests that by affecting water balance, cold hardiness, tolerance to wind, and by predisposing
19 plants to insect and disease pests, O₃ may even influence the occurrence and impact of natural
20 disturbance. Despite the probable occurrence of such effects, however, there are essentially no
21 instances where highly integrated ecosystem-level studies have conclusively shown that O₃ is
22 indeed altering ecosystem structure and/or function.

23 Systematic injury surveys demonstrate that foliar injury occurs on O₃-sensitive species in
24 many regions of the United States. However, the frequent lack of correspondence between foliar
25 symptoms and growth effects means that other methods must be used to estimate the regional
26 effects of O₃ on tree growth rates. Investigations of the radial growth of mature trees, in
27 combination with data from many controlled studies with seedlings, as well as a few studies with
28 mature trees suggest that ambient O₃ is reducing the growth of mature trees in some locations.
29 Studies using models based on tree physiology and forest stand dynamics suggest that modest
30 effects of O₃ on growth may accumulate over time and may interact with other stresses. For
31 mixed-species stands, such models predict that overall stand growth rate is generally not likely to

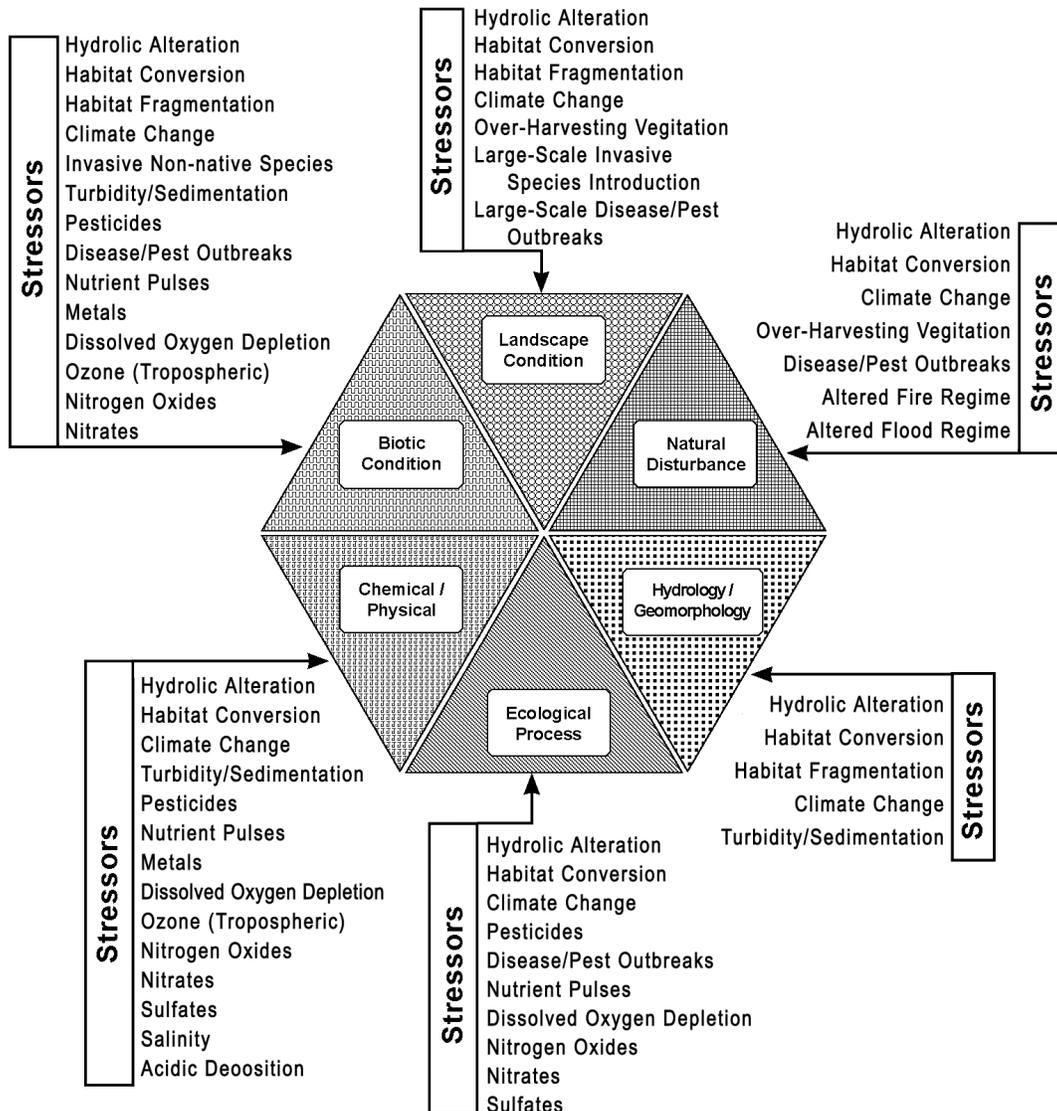


Figure AX9-21. Common anthropogenic stressors and the essential ecological attributes they affect.

Source: Modified from Young and Sanzone (2002).

- 1 be affected. However, competitive interactions among species may change as a result of growth
- 2 reductions of sensitive species. These results suggest that O₃ exposure over decades may be
- 3 altering the species composition of forests in some regions.
- 4

1 RESEARCH NEEDS

2 The knowledge base for examining the range of ecological effects of O₃ on natural
3 ecosystems is growing, but significant uncertainties remain regarding O₃ effects at the ecosystem
4 level. For example, there is a need for information on the following ecosystem-level responses:

- 5 • *Ecosystem Processes.* Little is known about the effects of O₃ on water, C, and nutrient cycling, particularly at the stand and community levels. Effects on belowground ecosystem processes in response to O₃ 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 O₃ on structural or functional components of soil food webs, or how these impacts could affect plant species diversity (Andersen, 2003).
- 6 • *Biodiversity and Genetic Diversity.* The study of genetic aspects of O₃ impacts on natural ecosystems has been largely correlational in nature and it remains to be shown conclusively whether O₃ affects biodiversity or genetic diversity (Davison and Barnes, 1998; Pitelka, 1988; Winner et al., 1991). Studies of competitive interactions under elevated O₃ 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 O₃ are needed. These studies could be strengthened by modern molecular methods to quantify impacts on diversity.
- 7 • *Natural Ecosystem Interactions with the Atmosphere.* Little is known about feedbacks between O₃ and climate change on volatile organic compound (VOC) production, which in turn, could affect O₃ production (Fuentes et al., 2001). At moderate-to-high O₃ 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 O₃ exposure effects, if included in the modeling effort have assumed a linear relationship between assimilation and stomatal conductance.
- 8 • *Below-Ground Interactions.* While the negative effects of O₃ on below ground growth are well characterized, interactions of roots with the soil or microorganisms are not.
- 9 • *Other Interactive Effects.* Interaction studies with other components of global change (e.g., warming, increasing atmospheric CO₂, N deposition, etc.) or with various biotic stressors are needed to better predict complex interactions likely in the future (Laurence and Andersen, 2003). Whether O₃ will negate the positive effects of an elevated CO₂ 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 O₃ affect the progress of pest epidemics and insect outbreaks as concentrations increase is unclear (Ball et al., 1998).
- 10 • *Reproduction Effects.* Information concerning the impact of O₃ 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).

- 1 • *Comparative Extrapolation.* The vast majority of O₃ 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 O₃ 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).
- 2 • *Scaling-Up Issues.* Scaling the effects of O₃ 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 O₃ gradients, and extensive modeling efforts to project landscape-level, regional, national and international impacts of O₃. 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.
- 3 • *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 O₃ effects on natural ecosystems (Heck et al., 1998).

4
5
6

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1 **AX9.7 ECONOMIC EVALUATION OF OZONE EFFECTS ON**
2 **AGRICULTURE, FORESTRY AND NATURAL ECOSYSTEMS**

3 **AX9.7.1 Introduction**

4 The adverse consequences of ambient air pollutant exposures on vegetation, ecosystems,
5 and components of the material environment have been documented since the beginning of the
6 industrial revolution. Attempts to quantify the monetary damage and injury resulting from
7 tropospheric O₃ exposures to managed agriculture, forests, and natural ecosystems date back at
8 least to the 1950s.

9 Both methodological and data problems plagued early efforts to assess the monetary
10 damages of air pollution to crops and natural vegetation. Adams and Crocker (1989) discussed
11 the methodological issues, e.g., a lack of reliable data on effects from air pollutants on crop
12 yields or the failure to develop and apply appropriate economic models. Some of these problems
13 were remedied by the EPA's National Crop Loss Assessment Network (NCLAN) in the 1980s.
14 The EPA's NCLAN facilitated the performance of economic assessments by providing O₃-crop
15 yield data with which to estimate O₃-crop yield response functions (see Heagle [1988] for a
16 review of NCLAN procedures and findings). NCLAN also funded a series of economic
17 assessments that, along with subsequent economic assessments, documented substantial
18 economic damages to agriculture. (See Spash [1997] for a detailed review of economic
19 assessments, many of which used NCLAN data.)

20 Since the completion of the NCLAN program in the late 1980s, the number of economic
21 assessments of air pollution studies focusing on terrestrial ecosystems in general, and agriculture
22 in particular, has declined. For example, for the period of 1980 to 1990, 33 economic studies
23 of O₃ and other air pollutant effects on U.S. crops were published in peer-reviewed journal
24 outlets (Spash, 1997). However, in preparing this section of the current criteria document, only
25 four peer-reviewed economic assessments were found for the decade of 1991 to 2000 that
26 addressed vegetation in the United States. In addition, one peer-reviewed article (Kuik et al.,
27 2000) was found dealing with agriculture in the Netherlands. Recent interest in global climate
28 change and the potential effects of global warming on O₃ and other photochemical oxidants, has
29 renewed interest in the effects of air pollution on both managed and unmanaged terrestrial
30 ecosystems (Adams et al., 1998). In addition, concern is growing regarding the effects of air

1 pollutants on natural ecosystems and on the services they provide (Daily, 1997). Unfortunately,
2 this interest has not yet translated into additional peer-reviewed publications addressing O₃ or
3 other air pollutant effects on ecosystems.

4 This section of the current criteria document first discusses the availability of economic
5 information and its usefulness in forming environmental policy. Next, economic assessments of
6 air pollution effects and findings from the 1996 AQCD (U.S. Environmental Protection Agency,
7 1996) are discussed, followed by a synthesis of the limited literature available since the 1996
8 AQCD with respect to O₃ effects on agriculture, forestry, and ecosystems. Finally, limitations
9 and continuing uncertainties are reviewed. The most fundamental of these is the lack of
10 measurements of the economic effects of air pollution on natural ecosystems. Other issues
11 include the variability of performance in both managed and natural ecosystems under increased
12 climatic and air pollution variability as well as the challenges related to spatial and temporal
13 scales used in performing economic assessments. To date, this set of effects has been sparsely
14 addressed.

16 **AX9.7.2 The Measurement of Economic Information**

17 Economic science is an exercise in deductive logic in which testable hypotheses about the
18 behavior of economic agents (i.e., farmers, consumers, resource owners) and markets are
19 deduced from a body of theory. That body of theory is based on a series of premises proposed
20 by economists and philosophers dating back over two centuries to Adam Smith. These premises
21 gradually evolved into a theoretical foundation primarily dealing with microeconomics and
22 culminating in structural relationships that define the operation of markets. This foundation was
23 first laid out in a comprehensive and rigorous fashion by Alfred Marshall in 1920. Samuelson
24 (1948) formalized these theoretical relationships, resulting in what is sometimes referred to as
25 modern, or neoclassical, economics.

26 The insights gained from the theoretical foundation of economics helped shape the nature
27 of applied economic, or policy-relevant research. An example of such applied research is when
28 economists seek to measure the economic consequences of air pollution on agriculture. Such an
29 application is described in Adams and Horst (2003), who provided a graphical representation of
30 the measurement of the effects of air pollution on the well-being of producers and consumers.

1 Economic theory is applied to real-world problems when the methods of economics and the need
2 for data from other disciplines come into play. When measuring the economic effects of an
3 environmental change, economists need an economic model or method that is theoretically
4 consistent, i.e, defensible, as well as data with which to estimate both economic and
5 environmental science relationships for use in the model. Among accepted economic assessment
6 methods, the actual choice is frequently determined by the nature of the problem to be addressed.
7 It should be noted that the choice of assessment method can affect the type of economic
8 information that is obtained. Even with a given assessment method, results are sensitive to
9 specific data treatment or assumptions (Adams, 1999). For example, some methods only
10 measure effects on a particular group e.g., farmers. Other methods may measure effects across
11 several groups. Thus, one should not expect the magnitudes of damage or benefits to be
12 identical across economic assessments. One should, however, expect that the direction of the
13 effects will be similar.

14 Once it is established that an assessment meets basic economic criteria, e.g., including
15 human behavioral responses, the selection of the specific economic assessment method is often a
16 relatively minor issue in terms of estimating benefits of air pollution control (or disadvantages of
17 increases in air pollution). Although results differ across approaches, the differences are largely
18 attributable to specific features of the assessment (e.g., whether the natural science data include a
19 particular effect or relationship, whether effects on consumers are measured, and so forth). The
20 nature and quality of the air quality forecasts used in the assessments can greatly influence the
21 sensitivity of the assessments (Adams and Crocker, 1989). This is particularly noticeable when
22 dealing with forecasts of seasonal air pollution changes (Adams et al., 1988). From the
23 standpoint of providing policy guidance, the differences in economic estimates attributable to the
24 assessment methods are often swamped by uncertainty in the natural and physical science
25 forecasts. This has also been noted in recent economic assessments of climate change (Adams
26 et al., 1998). In many settings, the quality of economic assessments of air pollution is likely to
27 be improved more by refining the physical and natural science data used in the assessments than
28 by intensive efforts to fine-tune the assessment techniques (Adams, 1999).

AX9.7.3 Understanding of Air Pollutants Effects on the Economic Valuation of Agriculture and Other Vegetation in the 1996 Criteria Document

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 that 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 is 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.

1 **AX9.7.3.1 Agriculture**

2 In view of the importance of U.S. agriculture for both domestic and world consumption of
3 food and fiber, reductions in U.S. crop yields could adversely affect human welfare. The
4 plausibility of this premise has resulted in numerous attempts to assess, in monetary terms, the
5 losses from ambient O₃ exposures, or the benefits of O₃ control, to agriculture. Twenty-three
6 assessments of the economic effects of O₃ exposures on agriculture were reviewed in the 1996
7 AQCD, highlighting key issues in the validity of these assessments (U.S. Environmental
8 Protection Agency, 1996). First, the evidence should reflect how crop yields will respond under
9 actual field conditions to O₃ exposures. Second, the air quality data used to frame current or
10 hypothetical effects of O₃ on crops should represent actual exposures sustained by crops at
11 individual sites or production areas. Finally, the assessment methodology into which such data
12 are entered should (1) capture the economic behavior of producers and consumers as they
13 adjust to changes in crop yields and prices that may accompany changes in O₃ air quality;
14 (2) accurately reflect institutional considerations, such as regulatory programs and income
15 support policies (e.g., provisions of federal Farm Bill legislation), that may result in market
16 distortions; and (3) use measures of well-being that are consistent with economic principles.

17 Assessments of O₃ damage to agricultural crops reported in the 1996 AQCD employed
18 procedures for calculating economic losses that met the conditions described above. More
19 specifically, the assessments provided 23 quantitative estimates of the economic consequences
20 of exposures to O₃ and other air pollutants to agriculture that reflect producer-consumer
21 decision-making processes, associated market adjustments, and some measure of distributional
22 consequences between affected parties. Many of the economic assessments reviewed in
23 previous O₃ documents also focused on O₃ effects in specific regions, primarily California and
24 the Corn Belt (e.g., Garcia et al., 1986). This regional emphasis in the earlier literature may be
25 attributed to the relative abundance of data on crop response and air quality for selected U.S.
26 regions, as well as the importance of some agricultural regions (such as California) for the U.S.
27 agricultural economy.

28 Two U.S. national studies described in previous O₃ criteria documents that are worthy of
29 additional comment are Kopp et al. (1985) and Adams et al. (1986). These were judged to be
30 “adequate” in terms of the three critical areas of data inputs in the 1996 AQCD. Together, it was
31 argued, they provide a reasonably comprehensive estimate of the economic consequences of

1 changes in ambient air O₃ levels on agriculture. Because of their central role in the 1996 criteria
2 document, these two studies are reviewed briefly below.

3 The Kopp et al. (1985) and Adams et al. (1986) studies indicated that ambient levels of O₃
4 were imposing substantial economic costs of ~\$3.4 billion (in 2000 U.S. dollars) on agriculture.
5 Both were judged to suffer from several sources of uncertainty, but the document concluded that
6 these possible improvements in future assessments were not likely to greatly alter the range of
7 agricultural benefit estimates arising from O₃ reductions for several reasons. First, the studies
8 covered about 75 to 80 % of U.S. agricultural crops (by value). For inclusion of the other 20%
9 to significantly change the estimates would require that their sensitivities to O₃ be much greater
10 than for the crops that have been included to date. Second, model-sensitivity analyses reported
11 in past studies indicate that changes in plant exposure response relationships must be substantial
12 to translate into major changes in economic benefits estimates. For example, it was assumed
13 unlikely that use of different exposure measures, or inclusion of interaction effects, would
14 greatly alter the magnitude of the economic estimates. Third, it was believed that countervailing
15 effects would mitigate against large swings in the estimates, e.g., longer O₃-exposure periods
16 may predict greater yield losses, but O₃-water stress interactions tend to reduce the
17 yield estimates.

18 Other national assessments reported in the 1996 AQCD provided general corroboration of
19 the results of Kopp et al. (1985) and Adams et al. (1986). An evaluation of these studies in
20 terms of the adequacy of information from plant exposure studies, and aerometric and economic
21 data was presented in the 1996 AQCD, along with estimates of benefits or damages associated
22 with changes in O₃. Most of the studies added onto either Kopp et al. (1985) or Adams et al.
23 (1986). A relevant question was whether subsequent studies provided any “surprises” in terms
24 of magnitude of economic effects.

25 Common themes or findings from these and earlier O₃ and other air pollution studies were
26 summarized in two synthesis papers, Adams and Crocker (1989) and Segerson (1991). The
27 major conclusion is that the agricultural effects of tropospheric O₃ at ambient levels impose
28 economic costs to society or, conversely, that reductions in ambient O₃ should result in societal
29 benefits.

30 Several studies contained in the 1996 AQCD still are of interest. For example, one finding
31 pertains to the relationship between federal farm programs and air pollution regulations

1 (McGartland, 1987). In each case, the inclusion of farm programs in the economic models
2 resulted in modest reductions in the economic benefits of O₃ control due to increased farm
3 program costs. As Segerson (1987) noted, however, it is not clear that these increased costs
4 should be charged against the potential benefits of an O₃-regulatory standard, but rather,
5 considered as an additional cost associated with the inefficiencies of the farm program. It should
6 also be noted that the nature of federal farm programs was changed dramatically by Congress in
7 1996 in an attempt to reduce the federal government's role in agriculture. Although more recent
8 federal legislation, such as the 2002 Farm Bill (U.S. Congress, 2002), appears to be restoring the
9 federal government's role in the farming sector, this issue currently is not as important as
10 suggested by earlier studies, due to the declining reliance on deficiency payments to farmers,
11 which tend to distort resource allocation.

12 Another national study (Adams et al., 1988) analyzed economic benefits under a regulatory
13 alternative involving a seasonal (crop growing season) O₃-exposure index measured as a 12-h
14 mean, instead of hourly levels or percent changes from ambient as reported in earlier studies.
15 Specifically, a seasonal average of 50 ppb O₃ (measured as a 12-h seasonal mean) with a
16 95% compliance level, is reported in Adams et al. (1988). The result (a \$2.9 billion benefit in
17 2000 dollars) is similar to the assumed 25% reduction across all regions reported by Adams et al.
18 (1986). At least one study also combined environmental stressors (e.g., O₃, UV-B radiation) in
19 performing economic assessments. Adams and Rowe (1990), using the same model as Adams
20 et al. (1986), reported that a 15% depletion of stratospheric O₃ (resulting in a 13% increase in
21 tropospheric O₃) would cause an economic loss of ~\$1.4 billion in 2000 dollars attributed to the
22 tropospheric O₃ increase. Reducing VOCs/NO_x motor vehicle emissions by 10% would result in
23 a benefit of ~\$0.3 billion, while a complete elimination of motor vehicle emissions would yield a
24 benefit of ~\$3.4 billion (1990 dollars). The range of these numbers is consistent with values
25 reported in Adams et al. (1986), Kopp et al. (1985), and other national-level analyses, i.e.,
26 estimates of from \$1.0 to 2.0 billion for reductions in ambient O₃ of 25 to 40%.

27 28 **AX9.7.3.2 Forests (Tree Species) and Natural Ecosystems**

29 The long-term nature of air pollution effects on perennial species such as trees creates
30 challenges to plant scientists in attempts to sort out the effects of specific individual stressors
31 such as O₃ from among the many other potential causal factors (Skelly, 1988). It also creates

1 problems in terms of measuring the impacts on direct economic value of goods, such as
2 reductions in board-feet of lumber produced per unit of time.

3 Most of the literature in the 1996 AQCD dealing with forest species reported the effects
4 of O₃ exposures in terms of foliar injury (Davis and Skelly, 1992; Freer-Smith and Taylor, 1992;
5 Simini et al., 1992; Taylor and Hanson, 1992). This emphasis on foliar effects in the forest
6 effects literature (rather than marketable yield) is similar to the state of science for agricultural
7 crops prior to 1980. More recent studies address the effects of air pollutants on forest tree
8 species diversity (Bringmark and Bringmark, 1995; Vacek et al., 1999; Weiner et al., 1997).
9 However, such information is of limited use in economic assessments. The exception is in
10 measuring the economic value of aesthetic changes in a forest stock, where changes in species
11 composition may affect recreational values (Crocker, 1985).

12 The data concerning changes in marketed output such as board-feet of lumber or changes
13 in growth rates in managed forests, the affects on the growth of almond, peach, apricot, pear and
14 plum trees in orchards cited in the 1996 document (U.S. Environmental Protection Agency,
15 1996) have not been quantified. In addition, the economic impact of reductions in growth of
16 seedlings of evergreen trees, e.g., slash pine, presented in the same document have not been
17 valued. The few studies which attempted to measure economic losses arising from exposures
18 to O₃ or other pollutants circumvented the lack of plant science data by assuming (often
19 arbitrary) reductions in forest species growth or harvest rates (Adams, 1986; Callaway et al.,
20 1986; Crocker and Forster, 1986; Haynes and Adams, 1992). Although the economic estimates
21 reported in the 1996 AQCD are comparable to those reported for agriculture (e.g., \$2.6 billion
22 for eastern Canada forests, \$2.9 billion for eastern U.S. forests in 2000 dollars), the lack of yield
23 and/or growth effects data makes these studies only suggestive at best, of the economic
24 consequences of forest effects directly attributable to O₃ exposures questionable. Recent
25 developments in forestry economic modeling capabilities, in support of climate change research,
26 have enhanced the ability to measure the effects of environmental stressors on this sector
27 (Adams et al., 1996; McCarl et al., 1998). However, these models need data on changes in either
28 timber production or growth rates, both of which are lacking for forest species under alternative
29 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/NO_x 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

1 little to the assessment of air pollution effects at the national level. Also, such reports are not
2 peer reviewed. Therefore, they are not discussed here.

3 There have been a number of recent studies of air pollutant effects on tree species in the
4 literature. Some have reported changes in total biomass and focused on European species
5 (Kurczynska et al., 1997). Other studies have assessed changes in composition of forest species
6 (biodiversity) or forest health due to exposure to air pollutants (Bringmark and Bringmark, 1995;
7 McLaughlin and Percy, 1999; Vacek et al., 1999). As noted previously, changes in forest
8 biomass and composition are more difficult to value than marketable products. However,
9 measures of forest composition or health have implications for an area of increasing policy
10 concern, that being the effect of air pollutants and other environmental stressors on unmanaged
11 (natural) ecosystems and the services they provide (Goulder and Kennedy, 1997; Pimentel et al.,
12 1997). Considerable discussion has occurred among ecologists and economists as to the
13 appropriate means for valuing these services (Anderson, 1990; Carpenter and Dixon, 1985;
14 Common and Perrings, 1992). A number of conceptual articles have been published on this
15 issue in both economic and ecological journals (Bergstrom, 1990; Castle, 1993; Pearce, 1993;
16 Suter, 1990).

17 A continuing empirical challenge concerns the lack of information on how changes in
18 biodiversity affect ecosystem performance resulting from O₃ stresses, and the problem of
19 establishing economic values for such changes (Cairnes and Lackey, 1992; Norton, 1987; Pimm,
20 1997; Polasky, 2001; Randall, 1991). As noted in the 1996 AQCD, and more recently by Daily
21 (1999, 2000) and Polasky (2001), there continues to be a lack of empirical studies that actually
22 assess the economic value of changes in biodiversity or in service flows due to any
23 environmental stressors. While some studies report monetary estimates, the estimates are
24 generally for expository purposes and those would not be as defensible as the agricultural studies
25 described earlier. For example, Costanza et al. (1997) assigned a value to the world's
26 ecosystems, but the procedures used render this an exploratory study at best. As assessed by
27 Polasky (2001), "In general, the field of valuation of ecosystem services is in its infancy."
28 He attributed the lack of empirical studies due to both a lack of the understanding of ecology of
29 ecosystem services and to the absence of reliable methods to estimate the value of these services.

30 In summary, the studies of crop and forest responses in the economic literature indicate
31 that O₃ reduces crop yields and imposes economic costs. The economic literature also indicates

1 that O₃ adversely influences the physiological performance of tree species and demonstrates, as
2 expected, that changes in growth have economic consequences. However, the economic data
3 and literature available on ecosystem effects of O₃ exposures are not sufficient to determine the
4 economic costs.

6 **AX9.7.5 Limitations of Scientific Studies and Economic Information**

7 The 1996 O₃ AQCD discussed the need for additional research on both ecological
8 functions and economic methodology in order to better understand the economic implications of
9 air pollutants on ecosystem services. As noted by Daily (1999, 2000), Polasky (2001), and
10 others, this research agenda continues to be important. Despite the large number of discussion
11 and survey articles published since 1996, there still are not sufficient data by which to estimate
12 confidently the magnitude of economic effects of O₃ to forests and natural ecosystems. Nor is it
13 apparent that ongoing research is adequate to answer this question in the next criteria document
14 cycle. Specifically, there do not appear to be any comprehensive, ecological studies underway
15 that attempt to measure changes in ecosystem outputs under alternative O₃ or other air pollutant
16 levels. Thus, in the near term, ecosystem services can only be discussed in qualitative terms.
17 However plausible the likelihood of economic damages to ecosystems, the available scientific
18 and economic information does not provide specific guidance on the magnitudes of these effects.

19 Beside the need to improve our understanding of the effects of O₃ exposures on natural
20 ecosystems, a number of areas of research could help assess the full economic consequences of
21 such pollutants on managed ecosystems. The first of these is the relationship between O₃
22 exposure levels and the variability of crop yields or changes in forest biodiversity. Most
23 assessments are based on the average or expected yield response of a crop to air pollution
24 exposure. However, the variability in yields (the spread or dispersion around the mean) appears
25 to be affected by the nature of plant exposure to pollutants (Hogsett et al., 1985; Musselman
26 et al., 1983). Plants exposed to the same mean dose but with different second moments of the
27 distribution of exposure may have different mean yields (Altshuller and Lefohn, 1996; Lefohn
28 and Benedict, 1982). In addition, the variability of the yield of the plant may also be increased
29 by greater variability in exposures (e.g., a higher frequency of extreme events). The economic
30 significance of higher yield varieties is such that variability may impose additional economic
31 costs, because most farmers have been reported to be averse to risk and prefer less variability for

1 a given yield (or profit). To date, no economic assessments of O₃ damages to agriculture or
2 vegetation in general include risk-averse behavior (studies cited here assumed risk neutrality).
3 To assess the economic consequences of a relationship between farmers' risk preferences
4 and O₃-induced changes in yield variability will require more information on the potential
5 effects of changes in O₃ on crop yield variability. While no economic studies were found on the
6 effects of O₃-induced changes in yield variability, a number of recent studies of climate change
7 effects on crop yields have indicated increased economic costs in the presence of increased
8 climatic variability (e.g., Mearns et al., 1997; Dixon and Segerson, 1999). Analogous economic
9 costs would be expected for changes in air pollution distributions; and these effects need to be
10 examined and quantified.

11 Another research area concerns the need improvement in our understanding of temporal
12 (dynamic) and spatial characteristics of O₃ exposures and their implications for crop yields,
13 production and producer profits. Most economic studies are static, in that they compare two
14 states of the world (e.g., economic activity at one O₃ level versus at an alternative level).
15 In addition, most national-level studies (the type needed to evaluate Secondary National
16 Ambient Air Quality Standards [SNAAQs]) display coarse regional-level resolution in terms of
17 crop response, O₃ exposure, and economic behavior. The responses of producers to changes in
18 yields due to changes in O₃ levels are generally assumed to be similar over geographical areas up
19 to several states in size. However, the changes between air quality scenarios are more likely to
20 be characterized by transient changes in exposure levels, which means the producer responses
21 are also likely to be gradual, rather than abrupt. Similarly, the lack of finer scale (regional-level)
22 data and modeling capabilities suggests that important micro-level physical and economic effects
23 are ignored. To what extent these abstractions influence net economic effects is an empirical
24 question. Research on these types of abstractions and assumptions within other economic
25 settings, such as climatic change, have shown that they have implications for economic
26 measurements (Adams, 2002).

27 Another issue is the natural or background level of O₃ (or other pollutants of interest)
28 assumed in economic studies. While many economic studies focus on changes in pollution
29 levels from current conditions, some studies have measured the economic damages between an
30 assumed, or pristine, level and current levels in agricultural areas. Such an analysis, it is
31 reasoned, will provide a measure of the net damages due to anthropogenic sources. The

1 challenge here is to have a correct measure of the background level of the pollutant. Recent
2 research by Lefohn et al. (2001) has suggested that background levels may be considerably
3 higher than assumed in some of the previous economic assessments reported in the 1996 AQCD
4 (25 to 30 ppb in most studies). For example, Lefohn et al. (Lefohn et al., 2001) detected hourly
5 readings of from 40 to 80 ppb during winter and spring months in remote areas of the United
6 States. If background O₃ levels are in the range, then the economic damages estimated in studies
7 with lower background levels will be overstated. The issue of the background O₃ level is
8 important to all assessments of vegetative damages due to O₃.

9 In terms of expanding economic methods for future assessment, analysts should consider
10 using more “reduced form” estimation methods, particularly in situations where the availability
11 of dose-response functions is limited. This estimation approach is exemplified by Garcia et al.
12 (1986). Specifically, their econometric study of the impact of O₃ on producer profits used
13 such a reduced-form approach. In this approach, farmer actions are modeled as a function of
14 ambient O₃ levels (along with other explanatory variables) without the direct use of dose
15 response functions. The advantage of this procedure is that one source of modeling uncertainty,
16 the need for dose-response functions, including time-consuming crop experiments to generate
17 data, is reduced. Actual responses of farmers’ profits across air pollution gradients of ambient
18 pollution are observed instead of hypothesized. Although this procedure has not been widely
19 used in air pollution economic assessments, it has been used in a number of relatively recent
20 climate change studies (e.g., Mendelsohn et al., 1994). The reduced-form method suffers from
21 the fact that if proposed O₃ levels are lower than those observed in the estimation sample, then
22 the prediction accuracy of the method deteriorates. Also, some dose-response information is
23 needed, if only to establish the plausibility of the economic estimates.

24 Another area that may improve economic assessments is incorporating consideration of
25 livestock issues. To date, most agricultural economic assessments of O₃ impacts have ignored
26 the livestock sector. Presumably this is because ambient O₃ levels do not noticeably affect meat
27 yields. However, if feed prices and pasture conditions are affected by ambient O₃ levels, then a
28 more accurate estimate of economic impacts would be forthcoming by including this link to
29 livestock in the assessment. These types of feed production and feed price effects are included
30 in the mathematical programming model used in Adams et al. (1986), but not in most other O₃
31 effects assessments. The significance of livestock-feed linkages are demonstrated in a recent

1 study in the Netherlands by Kuik et al (2000). Using a mathematical programming model
2 similar to that in Adams et al. (1986), Kuik et al. (2000) found that livestock effects were
3 prominent, due mainly to improved pasture yields under reduced ambient O₃ levels.
4

5 **AX9.7.6 Conclusions**

6 Substantial progress has been made over the past two decades in our understanding of the
7 effects of O₃ and other oxidants on vegetation, particularly for agriculturally important plant
8 species. The physical and economic effects on agriculture are well documented and provide
9 useful information for the setting of SNAAQs. Effects on forests and natural ecosystems remain
10 problematic, due to limitations in natural science data and economic methods. The problem is
11 most acute for valuing natural ecosystem service flows.

12 The current limitations surrounding forests and natural ecosystems present a rich research
13 agenda. However, not all research needs are likely to lead to better policies. Thus, areas of
14 greatest potential value in terms of regional policymaking need to be prioritized. Such priority
15 setting can be assisted by sensitivity analyses with existing economic models. By measuring the
16 changes in economic effects arising from changes in key parameters, research data gaps most
17 likely to affect economic values can be identified.
18
19

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