United States
Environmental Protection
Agency

February 2006
EPA 600/R-05/004cF

Air Quality Criteria for Ozone and Related Photochemical Oxidants

Volume III of III

EPA 600/R-05/004cF
February 2006

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
Research Triangle Park, NC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. 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 (O_3) in 1979 and a 1-hour O_3 NAAQS was set. The 1978 document focused mainly on the air quality criteria for O_3 and, to a lesser extent, on those for other photochemical oxidants (e.g., hydrogen peroxide and the peroxyacyl nitrates), as have subsequent revised versions of the document.

To meet Clean Air Act requirements noted above for periodic review of criteria and NAAQS, the O_3 criteria document, *Air Quality Criteria for Ozone and Other Photochemical Oxidants*, was next revised and released in August 1986; and a supplement, *Summary of Selected*

New Information on Effects of Ozone on Health and Vegetation, was issued in January 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O_3 was not appropriate at that time. That decision, however, did not take into account newer scientific data that had become available after completion of the 1986 criteria document. Such literature was assessed in the next periodic revision of the O_3 air quality criteria document (O_3 AQCD) which has completed in 1996 and provided scientific bases supporting the setting by EPA in 1997 of the current 8-h O_3 NAAQS.

The purpose of this revised air quality criteria document for O_3 and related photochemical oxidants is to critically evaluate and assess the latest scientific information published since that assessed in the above 1996 O_3 AQCD, with the main focus being on pertinent new information useful in evaluating health and environmental effects data associated with ambient air O_3 exposures. However, other scientific data are also discussed in order to provide a better understanding of the nature, sources, distribution, measurement, and concentrations of O_3 and related photochemical oxidants and their precursors in the environment. The document mainly assesses pertinent literature published through 2004, but also includes assessment of a few additional important studies published or accepted for publication in 2005.

A First External Review Draft of this O_3 AQCD (dated January 2005) was released for public comment and was reviewed by the Clean Air Scientific Advisory Committee (CASAC) in May, 2005 to obtain. Public comments and CASAC recommendations were then taken into account in making revisions to the document for incorporation into a Second External Review Draft (dated August, 2005), which underwent further public comment and CASAC review at a December, 2005 public meeting. Public comments and CASAC advice derived from review of that Second External Review Draft were considered in making revisions incorporated into this final version of the document (dated February, 2006). Evaluations contained in the present document will be drawn on to provide inputs to associated O_3 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 O_3 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 O_3 in outdoor (ambient) and indoor environments. It also evaluates the latest data on human exposures to ambient O_3 and consequent health effects in exposed human populations, to support decision making regarding the primary, health-related O_3 NAAQS. Lastly, the document also evaluates ambient O_3 environmental effects on vegetation and ecosystems, surface level solar UV radiation flux and global climate change, and man-made materials to support decision making on secondary O_3 NAAQS.

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

Air Quality Criteria for Ozone and Related Photochemical Oxidants

VOLUME I

Air Quality Criteria for Ozone and Related Photochemical Oxidants

(cont'd)

VOLUME II

VOLUME III

Table of Contents

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

List of Tables

List of Tables
(cont'd)

List of Figures

List of Figures
(cont'd)

Authors, Contributors, and Reviewers

CHAPTER 9 ANNEX (ENVIRONMENTAL EFFECTS)

Principal Authors

Dr. Jay Garner—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 (retired

Dr. Timothy Lewis—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. William Hogsett—National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR

Dr. Christian Andersen—National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR

Dr. Allen Lefohn—ASL and Associates, Helena, MT

Dr. David Karnosky—Forest Resources and Environmental Sciences, Michigan Technological University, Houghton, MI

Dr. Michael Nannini—Center for Aquatic Ecology, Illinois Natural History Survey, Kinmundy, IL

Dr. Nancy Grulke—Pacific Southwest Research Station Forest Fire Laboratory, USDA Forest Service, Riverside, CA

Dr. Richard Adams—Department of Agriculture and Resource Economics, Oregon State University., Corvallis, OR

Dr. Robert Heath—Department of Botany and Plant Sciences, University of California, Riverside, CA

Dr. Victor Runeckle—Biology Department, University of British Columbia, Vancouver, B.C., CN (Retired)

Dr. Arthur Chappelka—Auburn University, School of Forestry, Auburn, AL

Dr. William Massman—USDA Forest Service, Ft. Collins, CO

Dr. Robert Musselman—USDA Forest Service, Fort Collins, CO

Dr. Peter Woodbury—Cornell University, Ithaca, NY (former USDA Forest Service)

Authors, Contributors, and Reviewers
(cont'd)

Contributors and Reviewers

Dr. Fitzgerald Booker—USDA-ARS Plant Science Research Unit, 3908 Inwood Rd., Raleigh, NC 27603

Dr. Boris Chevone—Department of Plant Pathology, Virginia Technological University, Blacksburg, VA 24061

Dr. Alan Davison—School of Biology, Newcastle University, Newcastle on Tyne, United Kingdom, NE1 7RU

Dr. Bruce L. Dixon—Department of Agricultural Economics, University of Arkansas, Fayetteville, AR 72701

Dr. David Grantz—Kearney Agricultural Center, University of California at Riverside, Parlier, CA 93648

Dr. Allen S. Heagle—1216 Scott Pl., Raleigh, NC 27511

Dr. Robert Horst, Jr.—121 Thorwald Dr., Plainsboro, NJ 08536

Dr. John Innes—Forest Sciences Centre, Department of Forest Resources, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Dr. Hans-Jürgen Jäger—Heinrich-Buff-Ring 26-32, Institute of Plant Ecology, Justus-Leibig University, Gessen, Germany D35392

Dr. Robert Kohut— Tower Road, Boyce Thompson Institute, Rm 131,Cornell University, Ithaca, NY 14853

Dr. Sagar Krupa—1519 Gortner Ave., Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108

Dr. William Manning—203 Morrill, Department of Microbiology, University of Massachusetts, Amherst, MA 01003

Dr. Paul Miller—USDA Forest Service, Pacific Southwest Research Station, 4955 Canyon Crest Drive, Riverside CA 92507

Dr. Howard Neufeld—Rankin Science Bldg., Appalachian State University, Boone, NC 28608

Contributors and Reviewers
(cont'd)

Dr. Maria-Jose Sanz—Fundacion CEAM, c/Charles Darein, 14-Parque Te Valencia, Spain

Dr. James Shortle—Department of Ag Econ, Armsby, Pennsylvania State University, University Park, PA 16802

Dr. John Skelly—Department of Plant Pathology, Pennsylvania State University, University Park, PA 16803

U.S. Environmental Protection Agency Project Team for Development of Air Quality Criteria for Ozone and Related Photochemical Oxidants

Executive Direction

Dr. Lester D. Grant (Director)—National Center for Environmental Assessment-RTP Division, (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Scientific Staff

Dr. Lori White(Ozone Team Leader)—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. Joseph Pinto—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Ms. Beverly Comfort—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. Brooke Hemming—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. James S. Brown—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. Dennis Kotchmar—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. Jee-Young Kim—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. David Svendsgaard—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. Srikanth Nadadur—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. Timothy Lewis—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Dr. Jay Garner—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

U.S. Environmental Protection Agency Project Team for Development of Air Quality Criteria for Ozone and Related Photochemical Oxidants

(cont'd)

Scientific Staff
(cont'd)

Dr. William Hogsett—National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR

Dr. Christian Andersen—National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR

Mr. Bill Ewald—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 (retired)

Mr. James Raub—National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 (retired)

Technical Support Staff

Ms. Nancy Broom—Information Technology Manager, National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Mr. Douglas B. Fennell—Technical Information Specialist, National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Ms. Emily R. Lee—Management Analyst, National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Ms. Diane H. Ray—Program Specialist, National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Ms. Donna Wicker—Administrative Officer, National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 (retired)

Mr. Richard Wilson—Clerk, National Center for Environmental Assessment (B243-01), U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

U.S. Environmental Protection Agency Project Team for Development of Air Quality Criteria for Ozone and Related Photochemical Oxidants

(cont'd)

Document Production Staff

Ms. Carolyn T. Perry—Manager, Computer Sciences Corporation, 2803 Slater Road, Suite 220, Morrisville, NC 27560

Mr. John A. Bennett—Technical Information Specialist, Library Associates of Maryland, 11820 Parklawn Drive, Suite 400, Rockville, MD 20852

Mr. William Ellis—Records Management Technician, InfoPro, Inc., 8200 Greensboro Drive, Suite 1450, McLean, VA 22102

Ms. Sandra L. Hughey—Technical Information Specialist, Library Associates of Maryland, 11820 Parklawn Drive, Suite 400, Rockville, MD 20852

Mr. Matthew Kirk—Graphic Artist, Computer Sciences Corporation, 2803 Slater Road, Suite 220, Morrisville, NC 27560

Dr. Barbara Liljequist—Technical Editor, Computer Sciences Corporation, 2803 Slater Road, Suite 220, Morrisville, NC 27560

Ms. Rosemary Procko—Senior Word Processor, TekSystems, 1201 Edwards Mill Road, Suite 201, Raleigh, NC 27607

Ms. Faye Silliman—Publication/Graphics Specialist, InfoPro, Inc., 8200 Greensboro Drive, Suite 1450, McLean, VA 22102

Mr. Carlton Witherspoon—Graphic Artist, Computer Sciences Corporation, 2803 Slater Road, Suite 220, Morrisville, NC 27560

U.S. Environmental Protection Agency Science Advisory Board (SAB)
Staff Office Clean Air Scientific Advisory Committee (CASAC)
Ozone Review Panel

Chair

Dr. Rogene Henderson*, Scientist Emeritus, Lovelace Respiratory Research Institute, 2425 Ridgecrest Drive SE, Albuquerque, NM, 87108, Phone: 505-348-9464, Fax: 505-348-8541, (rhenders@lrri.org) (FedEx: Dr. Rogene Henderson, Lovelace Respiratory Research Institute, 2425 Ridgecrest Drive SE, Albuquerque, NM, 87108, Phone: 505-348-9464)

Members

Dr. John Balmes, Professor, Department of Medicine, University of California San Francisco, University of California - San Francisco, San Francisco, California, 94143, Phone: 415-206-8953, Fax: 415-206-8949, (jbalmes@itsa.ucsf.edu)

Dr. Ellis Cowling*, University Distinguished Professor-at-Large, North Carolina State University, Colleges of Natural Resources and Agriculture and Life Sciences, North Carolina State University, 1509 Varsity Drive, Raleigh, NC, 27695-7632, Phone: 919-515-7564 , Fax: 919-515-1700, (ellis_cowling@ncsu.edu)

Dr. James D. Crapo*, Professor, Department of Medicine, National Jewish Medical and Research Center. 1400 Jackson Street, Denver, CO, 80206, Phone: 303-398-1436, Fax: 303-270-2243, (crapoj@njc.org)

Dr. William (Jim) Gauderman, Associate Professor, Preventive Medicine, University of Southerm California, 1540 Alcazar #220, Los Angeles, CA, 91016, Phone: 323-442-1567, Fax: 323-442-2349, (jimg@usc.edu)

Dr. Henry Gong, Professor of Medicine and Preventive Medicine, Medicine and Preventive Medicine, Keck School of Medicine, University of Southern California, Environmental Health Service, MSB 51, Rancho Los Amigos NRC, 7601 East Imperial Highway, Downey, CA, 90242, Phone: 562-401-7561, Fax: 562-803-6883, (hgong@ladhs.org)

Dr. Paul J. Hanson, Senior Research and Development Scientist , Environmental Sciences Division, Oak Ridge National Laboratory (ORNL), Bethel Valley Road, Building 1062, Oak Ridge, TN, 37831-6422, Phone: 865-574-5361, Fax: 865-576-9939, (hansonpz@comcast.net)

Dr. Jack Harkema, Professor, Department of Pathobiology, College of Veterinary Medicine, Michigan State University, 212 Food Safety & Toxicology Center, East Lansing, MI, 48824, Phone: 517-353-8627, Fax: 517-353-9902, (harkemaj@msu.edu)

U.S. Environmental Protection Agency Science Advisory Board (SAB)
Staff Office Clean Air Scientific Advisory Committee (CASAC)
Ozone Review Panel
(cont'd)

Members
(cont'd)

Dr. Philip Hopke, Bayard D. Clarkson Distinguished Professor, Department of Chemical Engineering, Clarkson University, Box 5708, Potsdam, NY, 13699-5708, Phone: 315-268-3861, Fax: 315-268-4410, (hopkepk@clarkson.edu) (FedEx: 8 Clarkson Avenue, Potsdam, NY 136995708)

Dr. Michael T. Kleinman, Professor, Department of Community & Environmental Medicine, 100 FRF, University of California - Irvine, Irvine, CA, 92697-1825, Phone: 949-824-4765, Fax: 949-824-2070, (mtkleinm@uci.edu)

Dr. Allan Legge, President, Biosphere Solutions, 1601 11th Avenue NW, Calgary, Alberta, CANADA, T2N 1H1, Phone: 403-282-4479, Fax: 403-282-4479, (allan.legge@shaw.ca)

Dr. Morton Lippmann, Professor, Nelson Institute of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY, 10987, Phone: 845-731-3558, Fax: 845-351-5472, (lippmann@env.med.nyu.edu)

Dr. Frederick J. Miller*, Consultant, 911 Queensferry Road, Cary, NC, 27511, Phone: 919-467-3194, (fjmiller@nc.rr.com)

Dr. Maria Morandi, Assistant Professor of Environmental Science & Occupational Health, Department of Environmental Sciences, School of Public Health, University of Texas - Houston Health Science Center, 1200 Herman Pressler Street, Houston, TX, 77030, Phone: 713-500-9288, Fax: 713-500-9249, (mmorandi@sph.uth.tmc.edu) (FedEx: 1200 Herman Pressler, Suite 624)

Dr. Charles Plopper, Professor, Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, University of California - Davis, Davis, California, 95616, Phone: 530-752-7065, (cgplopper@ucdavis.edu)

Mr. Richard L. Poirot*, Environmental Analyst, Air Pollution Control Division, Department of Environmental Conservation, Vermont Agency of Natural Resources, Bldg. 3 South, 103 South Main Street, Waterbury, VT, 05671-0402, Phone: 802-241-3807, Fax: 802-241-2590, (rich.poirot@state.vt.us)

Dr. Armistead (Ted) Russell, Georgia Power Distinguished Professor of Environmental Engineering, Environmental Engineering Group, School of Civil and Environmental Engineering, Georgia Institute of Technology, 311 Ferst Drive, Room 3310, Atlanta, GA, 30332-0512, Phone: 404-894-3079, Fax: 404-894-8266, (trussell@ce.gatech.edu)

U.S. Environmental Protection Agency Science Advisory Board (SAB)
Staff Office Clean Air Scientific Advisory Committee (CASAC)
Ozone Review Panel
(cont'd)

Members
(cont'd)

Dr. Elizabeth A. (Lianne) Sheppard, Research Associate Professor, Biostatistics and Environmental & Occupational Health Sciences, Public Health and Community Medicine, University of Washington, Box 357232, Seattle, WA, 98195-7232, Phone: 206-616-2722, Fax: 206 616-2724, (sheppard@u.washington.edu)

Dr. Frank Speizer*, Edward Kass Professor of Medicine, Channing Laboratory, Harvard Medical School, 181 Longwood Avenue, Boston, MA, 02115-5804, Phone: 617-525-2275, Fax: 617-525-2066, (frank.speizer@channing.harvard.edu)

Dr. James Ultman, Professor, Chemical Engineering, Bioengineering program, Pennsylvania State University, 106 Fenske Lab, University Park, PA, 16802, Phone: 814-863-4802, Fax: 814-865-7846, (jsu@psu.edu)

Dr. Sverre Vedal, Professor of Medicine, Department of Environmental and Occupational Health Sciences, School of Public Health and Community Medicine, University of Washington, 4225 Roosevelt Way NE, Suite 100, Seattle, WA, 98105-6099, Phone: 206-616-8285, Fax: 206-685-4696, (svedal@u.washington.edu)

Dr. James (Jim) Zidek, Professor, Statistics, Science, University of British Columbia, 6856 Agriculture Rd., Vancouver, BC, Canada, V6T 1Z2, Phone: 604-822-4302, Fax: 604-822-6960, (jim@stat.ubc.ca)

Dr. Barbara Zielinska*, Research Professor , Division of Atmospheric Science, Desert Research Institute, 2215 Raggio Parkway, Reno, NV, 89512-1095, Phone: 775-674-7066, Fax: 775-674-7008, (barbz@dri.edu)

Science Advisory Board Staff

Mr. Fred Butterfield, CASAC Designated Federal Officer, 1200 Pennsylvania Avenue, N.W., Washington, DC, 20460, Phone: 202-343-9994, Fax: 202-233-0643 (butterfield.fred@epa.gov) (Physical/Courier/FedEx Address: Fred A. Butterfield, III, EPA Science Advisory Board Staff Office (Mail Code 1400F), Woodies Building, 1025 F Street, N.W., Room 3604, Washington, DC 20004, Telephone: 202-343-9994)

*Members of the statutory Clean Air Scientific Advisory Committee (CASAC) appointed by the EPA Administrator

ABBREVIATIONS AND ACRONYMS

AA	ambient air
ABA	abscisic acid
ABI2	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	family of cumulative, cutoff concentration-based exposure indices
APX	ascorbate peroxidase
AQCD	Air Quality Criteria Document
A_{sat}	photosynthetic assimilation in saturating light
ASC	ascorbate
ATPase	adenosine triphosphatase
AVG	1-aminoethoxyvinyl-glycine
AZO	azoxystrobin
BCB	blue copper binding protein
Cab	chlorophyll a/b binding protein
CAT	catalase
CEC	controlled environment chambers
cDNA	complementary DNA

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
CSTR	continuous stirred tank reactor
CU	cumulative uptake
CV	coefficient of variation
cyt	cytochrome
DG	diacylglycerol
DGDG	digalactosyldiacylglycerol
DHA	dehydroascorbate
DMPO	dimethylphrrolise 1-oxide; 5,5-dimethyl-1-pyrroline N-oxide
DNA	deoxyribonucleic acid
ECM	ectomycorrhizal fungi
EDU	ethylenediurea
EEA	essential ecological attribute
EMEP	European Monitoring and Evaluation Program
EPA	U.S. Environmental Protection Agency
EPO	epoxyconazole
EPR	electron paramagnetic resonance; ESR
ERD1	ethylene response
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
ϕPSII,max	maximum light-adapted apparent quantum efficiency of Photosystem II

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
G	plants rooted in ground
GDP	guanosine diphosphate
GGGT	galactolipid:galactolipid galactosyltransferase
GHG	greenhouse gas
GPx	glutathione peroxidase
GR	glutathione reductase
GRSM	Great Smoky Mountains National Park
GSH	glutathione
GSH-Px	glutathione peroxidase
GSMNP	Great Smoky Mountains National Park
GSSG	glutathione disulfide
GST	glutathione synthase
H^+	hydrogen ion
2HDM, 2ndHDM	second-highest daily maximum 1-h concentration
HF	hydrogen fluoride
HNO_3	nitric acid
H_2O_2	hydrogen peroxide
HO_3•	protonated ozone radical
HO•	hydroxyl radical
$HO_2^{\bullet}$	hydroperoxyl; hydroperoxy radical; protonated superoxide
HPOT	13-hydroperoxide linolenic acid
HR	hypersensitive response

ICP Forests	International Cooperative Programme on Assessment of Air Pollutant Effects on Forests
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
LOX1	lipoxygenase
M7	7-hour seasonal mean
mAOT	modified accumulated exposure over the threshold
MDA	malonaldehyde
MDGD	monogalactosyldiacylglycerol
MGDG	monogalactosyldiacylglycerol
mRNA	messenger ribonucleic acid
MT1	mitochondria
MV	methyl viologen
NAAQS	National Ambient Air Quality Standards
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
$(NH_4)_2SO_4$	ammonium sulfate
N_2O	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
$O_2^{\bullet}$	superoxide radical
$^1O_2$	singlet oxygen
O_3	ozone
OD	outer diameter
OTC	open-top chamber
p, P	probability value
P	plants grown in pots
PAD	pollutant applied dose
PAL	phenylalanine lyase
PAN	peroxyacetyl nitrate
PAR	photosynthetically active radiation
PC	phosphatidylchloline
PE	phosphatidylethanolamine
PFD	photosynthetic flux density
PG	phosphatidylglycerol
PGSM	Plant Growth Stress Model
PI	phosphatidylinositol

POD	peroxidase
ppb	parts per billion
ppm	parts per million
PQH_2	plastoquinone
PR	pathogenesis-related (protein)
PR-1	promotor region 1
PRYL	predicted relative yield (biomass) loss
PSII	Photosystem II
Pxase	peroxidase
qP	photochemical quenching
r^2	correlation coefficient
R^2	multiple regression correlation coefficient
rbcL	Rubisco large subunit
rbcS	Rubisco small subunit
RH	relative humidity
RNA	ribonucleic acid
ROG	reactive organic gases
ROS	reactive oxygen species
Rubisco	ribulose-1,6-P_2-carboxylase/oxygenase1
RuBP	ribulose bisphosphate
SA	salicylic acid
SAB	Science Advisory Board
SAG21	senescence
SAR	systemic acquired resistance
SD	standard deviation
SE	standard error
SHEN	Shenandoah National Park
SIGMOID	sigmoid weighted summed concentration

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 $\geq$ 0.06 ppm
SUM08	seasonal sum of all hourly average concentrations $\geq$ 0.08 ppm
TMPO	tetramethylphrrolise 1-oxide
TNC	total nonstructural carbohydrate
UDGT	UDP galactose-1,2,-diacylglycerol galactosyltransferase
UNECE	United Nations Economic Commission for Europe
UN ECE 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
UDP	uridine diphosphate
USDA	U.S. Department of Agriculture
UV	ultraviolet
UV-B	ultraviolet radiation of wavelengths from 280 to 320 nm
VOC	volatile organic compound
VPD	vapor pressure deficit
W95	cumulative integrated exposure index with a sigmoidal weighting function
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 O_3 on plant response ranges from subcellular to cellular, organismal, population, community, and ecosystem levels, with each level having its own particular experimental methodologies and specialized instrumentation, equipment, facilities, and experimental protocols. These investigations generate data. Other types of methodologies exist for the handling of data and statistical analysis as well as the utilization of data in developing the different exposure metrics or indices used to define exposure, quantitative exposure-response relationships, and computer simulation models of these exposure-response relationships. The objective of this section is not to provide an updated encyclopedia of all the methods that have been used but rather to focus on approaches that have

(1) led to an improved understanding of the quantitatively measurable growth and development responses of plants and plant communities to O_3, or

(2) provided information about the extent and geographic distribution of the responses of herbaceous and woody plants, both cultivated and native, to ambient O_3 exposures.

The first part of the objective is essential for determining dose-response functions used in developing impact and risk assessments of the effects of O_3; it usually involves treating plants to a range of artificial O_3 exposures. The second part of the objective is essential for determining the geographic distribution of the risk; it usually involves subjecting plants to ambient air O_3 exposures.

The types of methodologies used by biochemists, molecular biologists, or plant physiologists, whose interests lie in determining effects on specific constituents or in understanding the mode of action of O_3, are not discussed here but are addressed in Section AX9.2. Methods used to characterize the O_3 content of ambient air and to define exposure and exposure-response relations are discussed in Sections AX9.4 and AX9.5, respectively.

The methodologies for exposure-response studies have involved many different types of exposure facilities and protocols and have employed a range of statistical approaches in the analysis and interpretation of the data. Most of the studies have been conducted using major agricultural crop species. The methodologies have improved over the years as a result of the development, availability, or application of new or improved instrumentation, physical systems, and numerical approaches to data analysis. Yet equally important to the roles played by these advances has been the clearer understanding that has emerged from earlier work identifying the *type* of experimentation needed to achieve realistic assessments of the magnitude and extent of the impact of O_3 on vegetation of all types. As a result, significantly increased attention is now being paid to field observations and biomonitoring, particularly to the responses of forest trees and native vegetation.

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

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

AX9.1.2 Methods Involving Experimental Exposures to Ozone

AX9.1.2.1 "Indoor", Controlled Environment, and Greenhouse Chambers

The earliest experimental investigations of the effects of O_3 on plants utilized simple glass or plastic-covered chambers, often located within greenhouses, into which a flow of O_3-enriched air or oxygen could be passed to provide the exposure. The types, shapes, styles, materials of

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

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

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

The environmental conditions provided by indoor chambers of any type will always preclude the use of the information obtained with such chambers in predicting O_3 effects in the natural environment, because the environmental conditions will always be measurably different from field conditions. However, highly sophisticated controlled environment chambers such as those described by Langebartels et al. (1997), which are subdivided into aerial and root compartments with dynamic control of light intensity and photoperiod, air and soil temperature, humidity, soil moisture, wind speed, and exposure to O_3, may come close to simulating specific natural conditions. Such chambers have provided meaningful insights into a wide array of the early biochemical responses of plants to O_3. They can also minimize confounding factors that

make indoor chamber studies only rarely able to be extrapolated to field conditions, e.g., that shoots and roots develop under different temperature regimes. The applicability of the results of many chamber studies may be further limited by their use of container-grown plants. Most of the concerns over the applicability of CO_2 enrichment studies, as discussed in Section AX9.5.7.1, may also be relevant to O_3 enrichment studies, as suggested by Whitfield et al. (1996a).

Whitfield et al. (1996a) reported significant interactive effects between O_3 and soil volume on the growth of *Plantago major*. They noted that although container size may limit root and, hence, plant growth, the reverse may also be true for single plants in large containers, which do not experience typical field competition for resources. Other studies found little or no effect of rooting volume on plant response to O_3. Heagle et al. (1979a,b; 1983) found that four wheat cultivars (*Triticum aestivum* L.) had similar proportional suppression of seed yield to season-long O_3 exposure whether plants were grown in the ground or in 3.8-L pots. Similarly, proportional O_3 injury and yield response of field corn (*Zea mays* L.) (Heagle et al., 1979a) and soybean (*Glycine max* (L.) Merr.) (Heagle et al., 1983) was similar whether the plants were grown in 15-L pots or in the ground. In a two-year experiment with soybean, the relative effects of CO_2 and O_3 on above-ground biomass and seed yield were similar whether the plants were grown in pots (15 and 21 L) or grown in the ground (Booker et al., 2005). Collectively, the results suggest that while planting density and rooting environment affect plant morphology and growth, the relative responses of seed yield to elevated O_3 may be similar whether plants are grown in pots or in the ground.

AX9.1.2.2 Field Chambers

Although some types of closed field chambers have largely fallen out of favor in recent years, closed "Solardome" field chambers (Lucas et al., 1987; Rafarel and Ashenden, 1991) have been successfully used in studies of O_3 × acid mist interactions (Ashenden et al., 1995, 1996).

Concern over the need to establish realistic plant-litter-soil relationships as a prerequisite to studies of the effects of O_3 and CO_2 enrichment on ponderosa pine (*Pinus ponderosa*) seedlings led Tingey et al. (1996) to develop closed, partially environmentally controlled, sun-lit chambers ("terracosms") incorporating 1-m-deep lysimeters containing forest soil in which the appropriate horizon structure was retained.

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

Wiltshire et al. (1992) described a large OTC suitable for small trees with roll-up sides that permitted the trees to be readily subjected from time to time to episodic, normal, "chamberless" environmental conditions. In the 6-m-high OTCs described by Seufert and Arndt (1985) used with Norway spruce (*Picea abies*) trees, a second zone of annular enrichment was also provided between 4 and 5 m. The use of OTCs was adopted for the large European Stress Physiology and Climate Experiment on the effects of CO_2 and O_3 on spring wheat (ESPACE-wheat), conducted over 1994 to 1996 at field sites in eight countries (Jäger et al., 1999). However, typical European chambers have the introduction of O_3-enriched air at or above canopy height. The relatively low costs of fabrication, operation, and maintenance has favored OTC use in field studies (Fangmeier et al., 1992; Musselman and Hale, 1997). The air supplied to the chambers can be readily filtered through activated charcoal to reduce the O_3 concentration, or it can be enriched with O_3 to provide a range of exposures.

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

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

A second concern is that the introduction of the O_3-enriched air into the lower part of chambers as described by Heagle et al. (1973) and Mandl et al. (1973) results in a O_3 concentration gradient that decreases with increasing height, the converse of the situation observed in ambient air in which the O_3 concentration decreases markedly from above a plant canopy to ground level (Grünhage and Jäger, 1994; Pleijel et al., 1995b, 1996). Concern that studies conducted in such OTCs may somewhat overestimate the effects of O_3 led to the European design, which provides a decreasing downward gradient. It seems unlikely that the "chamber effects" produced by the two designs will be the same. These issues are discussed more fully in Section AX9.1.2.4.

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

The difficulties faced in the experimental exposure of forest trees to air pollutants in chambers (e.g., Seufert and Arndt [1985]) led to the development of branch chambers such as those described by Ennis et al. (1990), Houpis et al. (1991), and Teskey et al. (1991). These chambers are essentially large cuvettes without temperature control and, as noted by Musselman and Hale (1997), share many of the characteristics of CSTRs, i.e., transparent walls, internal fans, and inlet and outlet monitors to permit the determination of O_3 uptake, CO_2 exchange, and transpiration. Although they make it possible to expose whole branches to different O_3 regimes, the relevance of the data they yield in regard to the whole tree may be questionable. As noted by Saxe et al. (1998), the inevitable change in environmental conditions resulting from the isolation of the branch may cause different responses from those that would be obtained if the whole tree had been subjected to the same environmental conditions.

AX9.1.2.3 Plume Systems

Plume systems are chamberless exposure facilities in which the atmosphere surrounding plants in the field is modified by the injection of pollutant gas into the air above or around them from multiple orifices spaced to permit diffusion and turbulence so as to establish relatively homogeneous conditions as the individual plumes disperse and mix with the ambient air. As pointed out by Manning and Krupa (1992), they can only be used to *increase* the O_3 levels in the ambient air. The volume of air to be modified is unconfined, and three approaches have been used to achieve desired pollutant concentrations in the air passing over the plants, producing various systems that

(1) achieve a concentration gradient, in most instances dependent upon the direction of the prevailing wind;

(2) achieve spatially uniform concentrations over a plot, dependent upon wind direction; and

(3) seek to achieve spatially uniform concentrations over a plot, independent of wind speed and direction.

Gradient systems created by dispensing a pollutant gas into the air at canopy level from perforated horizontal pipes arranged at right angles to the prevailing wind were described for SO_2 studies in the early 1980s. A modified gradient system for O_3 was used by Bytnerowicz et al. (1988) to study effects on desert species, but there appear to have been no recent applications of the method. A gradient O_3-*exclusion* system is discussed in Section AX9.2.3.1.

Systems designed to achieve spatially uniform pollutant levels by ensuring that the release of a pollutant is always on the upwind side of the study site were also originally described for SO_2 studies (e.g., Greenwood et al. [1982]). However, the adaptation of these concepts as introduced by McLeod et al. (1985) in constructing a large circular field site for exposing crops to SO_2 led to the subsequent development of both the large-scale O_3 and SO_2 fumigation system for forest trees in the United Kingdom in 1985 (the Liphook Forest Fumigation Project) (1992), the smaller system for O_3 fumigation constructed at Kuopio, Finland in 1990 (Wulff et al., 1992), and the free-air carbon-dioxide enrichment (FACE) systems of gas dispersal over crops (Hendrey and Kimball, 1994) and forest trees (Hendrey et al., 1999). Although originally designed to provide chamberless field facilities for studying the CO_2 effects of climate change, large forest tree FACE systems have recently been adapted to include the dispensing of O_3

(Karnosky et al., 1999). Volk et al. (2003) recently described a system for exposing grasslands that uses 7-m diameter plots. FACE systems discharge the pollutant gas (and/or CO_2) through orifices spaced along an annular ring (or torus) or at different heights on a ring of vertical pipes. Computer-controlled feedback from the monitoring of gas concentration regulates the feed rate of enriched air to the dispersion pipes. Feedback of wind speed and direction information ensures that the discharges only occur upwind of the treatment plots, and that discharge is restricted or closed down during periods of low wind speed or calm conditions. The diameter of the arrays and their heights (25 to 30 m) in some FACE systems requires large throughputs of enriched air per plot, particularly in forest tree systems. The cost of the throughputs tends to limit the number of enrichment treatments, although Hendrey et al. (1999) argued that the cost on an enriched volume basis is comparable to that of chamber systems.

An alternative to the FACE system to free-air fumigation uses a horizontal grid system through which pollutant-enriched air is discharged over the canopies of plants in field plots. The original design, termed the Zonal Air Pollution System (ZAPS), was developed for studying the effects of SO_2 on native grasslands (Lee et al., 1975), and it was later modified by Runeckles et al. (1990) by randomly dividing each of three treatment plots into four subplots, each with different numbers of discharge orifices to provide various levels of O_3 enrichment. With the ZAPS system, changes in wind direction and speed result in varying degrees of carryover from subplot to subplot, effectively resulting in 12 stochastically different seasonal exposures. The system was used for studies of growth effects on field crops and 2- to 4-year old Douglas fir (*Pseudotsuga menziesii*) saplings (Runeckles and Wright, 1996). A larger ZAPS design was used by Wilbourn et al. (1995) on a grass (*Lolium perenne*)-clover (*Trifolium repens*) mixture and by Ollerenshaw et al. (1999) on oilseed rape (*Brassica napus*), whereby four replicate field plots were exposed to intermittent constant additions of O_3 to ambient air. A ZAPS design with eight spatially separated treatment plots was also developed to obtain crop response data used in assessing crop losses in the Fraser Valley, British Columbia, Canada (Runeckles and Bowen, 2000).

Another recent adaptation of the FACE design was constructed to fumigate soybean with CO_2 and O_3 in combination (Morgan et al., 2004; Rogers et al., 2004). This modified FACE design was based on those of Miglietta et al. (2001) and does not force air through the canopy; instead, it relies on wind to disperse air across the fumigation plot.

The FACE-type facility developed for the Kranzberg Ozone Fumigation Experiment (KROFEX) in Germany begun in 2000 (Werner and Fabian, 2002; Nunn et al., 2002) to study the effects of O_3 on mature stands of beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees is more truly a zonal system that functions independently of wind direction. The enrichment of a large volume of the ambient air immediately above the canopy takes place via orifices in vertical tubes suspended from a horizontal grid supported above the canopy.

Recognizing the difficulties of modifying the aerial environments of large trees, Tjoelker et al. (1994) devised a free-air system for exposing branches of sugar maple (*Acer saccharum*) trees to O_3. Near the ends of up to 10 branches, enriched air was discharged through small holes in 38-cm-diameter loops of 0.635-cm-OD (outer diameter) teflon tubes positioned 20 to 30 cm below the terminal foliage cluster.

Although plume systems make virtually none of the modifications to the physical environment that are inevitable with chambers, their successful use depends on selecting the appropriate numbers, sizes, and orientations of the discharge orifices to avoid hot-spots resulting from the direct impingement of jets of pollutant-enriched air on plant foliage (Werner and Fabian, 2002). However, because mixing is unassisted and completely dependent on wind turbulence and diffusion, local gradients are inevitable even in large-scale FACE systems. Both FACE and ZAPS systems have provisions for shutting down under low wind speed or calm conditions and for an experimental area that is usually defined within a generous border in order to strive for homogeneity of the exposure concentrations within the treatment area. They are also both dependent upon continuous computer-controlled feedback of the O_3 concentrations in the mixed treated air and of the meteorological conditions.

AX9.1.2.4 Comparative Studies

All experimental approaches to the exposure of plants to O_3 have shortcomings. The use of laboratory, greenhouse, or field chambers raises concerns for the roles of chamber effects on micrometeorology, as well as the constant turbulence over and within the plant canopy during chamber operation, in modifying O_3 uptake and subsequent plant response. In contrast, plume systems suffer from relatively poor control of exposure levels and an inability to reduce O_3 levels below ambient in areas where O_3 is phytotoxic.

Although chamber effects vary, one concern is the rise in temperature associated with enclosing plants in a chamber. Still, it is not clear whether these effects are directly related to temperature or are the result of temperature interactions with other environmental variables. For example, Olszyk et al. (1992) undertook a 3-year study of the impact of O_3 on Valencia orange trees (*Citrus sinesis* (L.) Osbeck) in large OTCs to determine if "insidious differences in microclimatic conditions could alter plant growth responses and susceptibility to pollutant stress." Nonfiltered chambers were found to have somewhat lower average O_3 concentrations than the ambient air, and fewer hourly exceedances of 100 ppb. In cool seasons, stomatal conductance was also lower, implying lower O_3 uptake. However, the cumulative fruit yields were doubled in the chamber trees even though photosynthetically active radiation was consistently reduced by about 19% while leaf temperatures averaged more than 2 °C higher. These data may be somewhat extreme, but they emphasize the need to be cautious when interpreting OTC yield response data, particularly since, as in this study, no O_3 enrichment was involved as a complicating factor.

While it is clear that chambers can alter some aspects of plant growth, the question to be answered is whether or not these differences affect plant response to O_3. As noted in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), evidence from the comparative studies of OTCs and from closed chamber and O_3-exclusion exposure systems on the growth of alfalfa (*Medicago sativa*) by Olszyk et al. (1986a) suggested that, since significant differences were found for fewer than 10% of the growth parameters measured, the responses were, in general, essentially the same regardless of exposure system used and chamber effects did not significantly affect response. In 1988, Heagle et al. (1988) concluded: "Although chamber effects on yield are common, there are no results showing that this will result in a changed yield response to O_3." A more recent study of chamber effects examined the responses of tolerant and sensitive white clover clones (*Trifolium repens*) to ambient O_3 in greenhouse, open-top, and ambient plots (Heagle et al., 1996). For individual harvests, greenhouse O_3 exposure reduced the forage weight of the sensitive clone 7 to 23% more than in OTCs. However, the response in OTCs was the same as in ambient plots. Several studies have shown very similar yield response to O_3 for plants grown in pots or in the ground, suggesting that even such a significant change in environment does not alter the proportional response to O_3, at least as long as the plants are well watered (Heagle, 1979; Heagle et al., 1983).

Recent evidence obtained using free-air exposure systems and OTCs supports results observed previously in OTC studies (Table AX9-16, Figure AX9-1). Specifically, a series of studies undertaken using free-air O_3 enrichment in Rhinelander, WI (Isebrands et al., 2000, 2001) showed that O_3-symptom expression was generally similar in OTCs, FACE, and ambient-O_3 gradient sites, supporting the previously observed variation among trembling aspen clones (*Populus tremuloides* L.) using OTCs (Karnosky et al., 1999). The FACE study evaluated the effects of 3 years of exposure to combinations of elevated CO_2 and O_3 on growth responses in mixture of five trembling aspen clones (Isebrands et al., 2000, 2001). Height, diameter, and stem volume (diameter^2 × height) were decreased by elevated O_3. On average for all clones, stem volume was decreased by 20% over the 3 years in the elevated O_3 treatment as compared with the 1×-ambient treatment. This FACE facility study is important, because it confirms responses reported previously with the same clones grown in pots or soil in OTCs without the alterations of microclimate induced by chambers. Currently, this is the only U.S. study using this technology to have examined the effects of O_3 under these conditions. This study is also significant, because the elevated O_3-exposure pattern used was intended to reproduce the 6-year average pattern from Washtenaw County, Michigan (Karnosky et al., 1999).

Chambered systems such as OTCs provide a charcoal-filtered (CF), clean-air control for O_3 experiments, while FACE and some other plume systems do not. Depending on experimental intent, a replicated, clean-air control treatment is an essential component in many experimental designs. This control cannot be provided by FACE systems where ambient O_3 levels are phytotoxic. This is especially relevant in CO_2 × O_3 experiments in which phytotoxic effects of ambient O_3 can be suppressed due to CO_2-induced reductions in stomatal conductance and O_3 uptake (Booker et al., 1997, 2004, 2005; Heagle et al., 1998b; Fiscus et al., 1997, 2002, 2005).

Plume systems avoid chamber effects, but because they rely solely upon diffusion and natural turbulence to modify the ambient O_3 concentration, they may fail to achieve homogeneity of the air to which the plants are exposed and may give rise to hot spots in which the enriched air jets are inadequately diluted and impinge directly on foliage. A further deterrent to their widespread use is the large-scale generation of O_3 needed, which has, in most cases, limited the numbers of treatments that can be included in an experimental design.

In spite of the various advantages and disadvantages of the two systems, there is still little experimental evidence that allows a direct comparison of OTCs to the free-air plume systems or a determination of the degree to which chamber effects alter plant response to O_3. The evidence that is available suggests that chamber effects do not fundamentally alter the response of plants to O_3; therefore, chambers remain a useful tool for testing species sensitivity and developing O_3-response relationships. However, chamber effects have the potential to alter O_3 uptake (Nussbaum and Fuhrer, 2000), so it is important to fully characterize temperature, light, turbulence, and other chamber characteristics during exposure to allow extrapolation of the results.

AX9.1.2.5 Ozone Generation Systems

Two approaches have been used to generate the O_3 needed for enrichment from air or oxygen: (1) high-voltage static discharge and (2) high-intensity UV-irradiation. Using gaseous oxygen as feedstock, both generate O_3-enriched oxygen, free from other impurities. However, the use of high-voltage discharge equipment with air as feedstock requires that the output be scrubbed with water to remove appreciable amounts of the higher oxides of nitrogen (especially nitric acid vapor) that form concurrently with O_3 (Brown and Roberts, 1988; Taylor et al., 1993).

AX9.1.2.6 Experimental Exposure Protocols

A few recent chamber studies of physiological or biochemical effects have continued to use square-wave exposure profiles typified by a rapid rise to, and falling off from, a steady target concentration. However, during the last 20 years, most approaches into studying O_3 effects on plant growth and development have employed either simulations of the diurnal ambient O_3 profile or enhancement/reduction of the ambient O_3 concentrations.

Hogsett et al. (1985b), Lefohn et al. (1986), and others have described the use in controlled chambers of daily exposure profiles based on observed ambient O_3 profiles. Such profiles were used in the elaborately controlled chamber studies of Langebartels et al. (1997), while several recent chamber studies have used simpler computer-controlled half- or full-cosine wave profiles to simulate the typical daily rise and fall in ambient O_3 levels (Mazarura, 1997; McKee et al., 1997a,b).

The early studies with OTCs involved adding constant levels of O_3 to ambient air O_3 concentrations, but all recent studies have used enrichment delivery systems that maintain proportionality to, and track, ambient O_3 concentrations to produce levels that more closely resemble field observations. Both FACE and ZAPS studies have used proportional enrichment to provide a range of treatments, although Wilbourn et al. (1995) and Ollerenshaw et al. (1999) adjusted their systems manually to obtain a relatively constant target concentration during exposure episodes.

AX9.1.3 Methods Involving Exposures to Ozone in Ambient Air

The experimental methods discussed above are largely aimed at developing quantitative growth-response functions to permit the estimation of the effects of different ambient O_3 scenarios. Because such methodologies usually involve exposures to higher than ambient O_3 levels, the applicability of the functions obtained may, to some extent, be relevant only to locations that are naturally subjected to high ambient O_3 levels. Furthermore, as pointed out in Section AX9.4, the response functions that they generate rarely incorporate other environmental, genetic, and physiological factors, many of which can severely modify the magnitude of the response to O_3. The consequences of ignoring such modifications have been well stated by De Santis (1999). The European level for protecting crops (based on the AOT40 index; see Section AX9.4) was derived from OTC studies of O_3-induced yield loss in wheat observed in experiments conducted mostly in non-Mediterranean locations. However, the impact of ambient O_3 on wheat yields in the Po Valley of northern Italy is much less than the devastatingly high loss (>60%) suggested by the seasonal exceedances of the level. On a similar note, Manning (2003) has recently urged the absolute necessity of seeking "ground truth" as verification of the nature and magnitude of impacts on vegetation as suggested by response functions using ambient O_3 monitoring data.

Such concerns clearly show that attention needs to be focused on incorporating consideration of environmental and other factors into the response functions upon which standards are based. This will require the development of improved simulation response models. These concerns have also led to increasing attention being paid to seeking and developing alternative approaches to assessing of impact, and the geographic extent of such impact—approaches that are based on in situ exposures to ambient or sub-ambient O_3 levels.

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

AX9.1.3.1 Air-Exclusion Systems

The term, air-exclusion system, usually refers to a chamberless field system specifically designed to protect plants from exposure to polluted air by blowing filtered air through their canopies. Hogsett et al. (1987a,b) described several dedicated systems developed in the 1960s and 1970s, but there appear to have been no recent O_3-exclusion studies using systems specifically designed for this purpose since those described by Olszyk et al. (1986a,b). Their system, a modification of the earlier system of Jones et al. (1977), consisted of perforated 31.8-cm OD inflatable polyethylene tubes laid between crop rows and supplied with CF air. By increasing the size of the orifices progressively in sections along the 9-m length of the tubes, an exclusion gradient was created with a progressive decrease in O_3 levels in the air surrounding the crop from one end of the system to the other. The system was used for studies on alfalfa comparing plant response in OTCs, closed field chambers, the air-exclusion system, and ambient air plots (as discussed above in Section AX9.1.2.4).

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

AX9.1.3.2 Natural Gradients

Naturally occurring O_3 gradients hold potential for the examination of plant responses along the gradient. However, few such gradients can be found which meet the rigorous statistical requirements for comparable site characteristics such as soil type, temperature, rainfall, radiation, and aspect (Manning and Krupa, 1992); although with small plants, soil variability can be avoided by the use of potted plants. The use of soil monoliths transported to various locations along natural O_3 gradients is another possible approach to overcome differences in soils; however, again this approach is limited to small plants.

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

A transect study of the impact of O_3 on the growth of white clover and barley (*Hordeum vulgare* L.) in the United Kingdom was confounded by differences in the concurrent gradients of SO_2 and NO_2 pollution (Ashmore et al., 1988). Studies of forest tree species in national parks in the eastern United States (Winner et al., 1989) revealed increasing gradients of O_3 and visible foliar injury with increased elevation.

AX9.1.3.3 Use of Chemical Protectants

The use of protective chemicals is a relatively inexpensive, promising alternative to experimental field exposures in chambers or free-air systems for determining plant response to O_3. These chemicals have recently been used in studies of different plant species, both in the United States (Bergweiler and Manning, 1999; Kuehler and Flagler, 1999) and in Europe (Bortier et al., 2001a; Pleijel et al., 1999; Wu and Tiedemann, 2002), to determine if ambient O_3 concentrations affect plant growth and productivity or are just exacerbating foliar injury. Several chemical compounds (e.g., antioxidants, antisenescence agents, fungicides, pesticides) have been known for many years to provide plants some protection from photochemical oxidants such as O_3 (Manning and Krupa, 1992). Most of these chemicals were originally used as a one-time application to reduce visible injury caused by acute O_3 exposures. The most widely used and popular of these has been ethylenediurea (EDU). Carnahan et al. (1978) reported that EDU protected pinto bean (*Phaseolus vulgaris*) from acute O_3 injury. After this initial investigation, EDU was shown to suppress visible O_3 injury on several species of plants under both controlled and field conditions (Brennan et al., 1987; Clarke et al., 1983). However, due to lack of a commercial market for this product, its commercial manufacture was largely discontinued. Other chemicals, including benomyl (Manning et al., 1974), carboxin (Rich et al., 1974), ascorbic acid (Dass and Weaver, 1968), and others (Manning and Krupa, 1992), also exhibited some beneficial effects in reducing visible O_3 injury.

Several recent studies have used EDU in assessing the response of several plant species to O_3 to help validate the proposed critical level (AOT40 = 3000 ppb·h; see Section AX9.5) for crop protection in Europe (Ball et al., 1998; Ribas and Penuelas, 2000; Tonneijck and Van Dijk, 2002a,b). EDU appeared to provide protection from visible foliar injury, but the results regarding yield and biomass reductions were mixed. In a 3-year study over 12 sites throughout Europe, Ball et al. (1998) used the ratio of EDU-treated versus non-treated white clover biomass

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

In another study, Tonneijck and Van Dijk (2002b) assessed the relationship of visible injury of subterranean clover (*Trifolium subterraneum*) to ambient O_3 at four sites over three growing seasons in the Netherlands, using EDU-treated and nontreated plants. Visible injury varied by site and year, but was reduced to near zero by EDU treatment. However, no relationship indicative of a protective effect of EDU with this plant species was observed for biomass. Tonneijck and Van Dijk (2002a) also reported similar results with pinto bean. Both EDU-treated and nontreated plants were exposed to ambient O_3 at three locations in Spain over one growing season (Ribas and Penuelas, 2000). Reductions in yield and biomass were correlated with O_3 concentration and EDU provided some protective effect, although results varied by location and with meteorological conditions.

Chemicals have also been used to assess the effects of O_3 on tree species. Bortier et al. (2001b) injected seedlings of an O_3-sensitive poplar (*Populus nigra*) clone with EDU and measured growth over a 1-year period at a field site near Brussels, Belgium. Over the growing season, stem diameter increment was significantly higher (16%), biomass was increased (9%), and foliar O_3 symptoms were slightly less for the EDU-treated seedlings. Ozone levels were reported to be low (AOT40 = 6170 ppb·h, May to September) during the exposure period. In another study, Manning et al. (2003) applied EDU (foliar spray) and sodium erythorbate (NaE) at various concentrations, biweekly for three growing seasons to loblolly pine (*Pinus taeda*) at a field site in east Texas. After 3 years, the trees were harvested and biomass measured. Neither EDU nor NaE prevented foliar O_3 injury, but EDU applications at 450 ppm resulted in increases both in stem diameter and height and in total above-ground biomass. These measures of growth also tended to slightly increase with applications of NaE, but the effects were statistically nonsignificant.

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

developed fungicides (azoxystrobin and epoxiconazole) provided protection to spring barley to relatively high O_3 exposures (150 to 250 ppb, 5 days, 7 h/day) and resulted in increases in leaf soluble protein content as well as the activity of several antioxidative enzymes (e.g., superoxide dismutase, catalase, ascorbate-peroxidase, and glutathione reductase). In addition, the increase in these enzymes reduced superoxide levels in the leaves.

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

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

Several studies suggest that EDU has effects other than its antioxidant protection and phytotoxicity and show that environmental conditions affect the degree of protection afforded by protective chemicals. In one study, even low concentrations of EDU (8 to 32 mg L^{-1} soil), decreased soybean yield under low O_3 exposure (7-h mean of 19 ppb) in CF OTCs (Miller et al., 1994). This study also demonstrated that phytotoxicity (both foliar symptoms and growth effects) can differ even in the same series of experiments, apparently due to changes in environmental conditions, and that EDU can suppress yield at application rates that do not always cause foliar symptoms (Miller et al., 1994). Finally, this study found that EDU altered biomass partitioning by increasing vegetative growth and decreasing reproductive growth.

A study of pinto bean grown in OTCs in Germany found that EDU treatment in CF OTCs significantly increased yield, while EDU had no significant effect on yield in other O_3 treatments (Brunschon-Harti et al., 1995). In this study, O_3 significantly reduced the mass of pods, shoots, and roots. EDU increased root, leaf, and shoot mass across O_3 treatments. However, the only statistically significant interaction occurred with O_3 × root mass. This study indicates that EDU can stimulate above-ground growth and/or delay senescence regardless of O_3 treatment.

The EDU approach for assessing the impact of ambient O_3 exposures is potentially useful, because it provides a separate line of evidence from other methods. Before using these chemicals in a field setting, preliminary investigations under controlled conditions (e.g., chambers) should be done to evaluate the methods and timing of application, as well as proper application rates, so as to avoid any potential toxic effects (Manning, 2000; Manning and Krupa, 1992). Unfortunately, such characterization has so far been limited, although substantial progress has been made for radish (*Raphanus sativus* L.) (Kostka-Rick et al., 1993; Kostka-Rick and Manning, 1992a, 1993). Thus, it is difficult to use data from existing EDU studies to develop exposure-response relationships or to quantify the effects of ambient O_3 exposure. Despite these limitations, the EDU studies reviewed in previous O_3 AQCDs (U.S. Environmental Protection Agency, 1986, 1996) and the more recent studies summarized in Table AX9-1 (Section AX9.5) provide another line of evidence that ambient O_3 exposures occurring in many regions of the United States may be reducing the growth of crops and trees.

AX9.1.3.4 Biomonitoring

Bioindicators

The use of biological indicators to detect the presence of O_3 injury to plants is a longstanding and effective methodology (Chappelka and Samuelson, 1998; Manning and Krupa, 1992). A bioindicator can be defined as a vascular or nonvascular plant exhibiting a typical and verifiable response when exposed to a plant stress such as an air pollutant (Manning et al., 2003). To be considered a good indicator species, plants must

(1) exhibit a distinct, verified response,

(2) have few or no confounding disease or pest problems, and

(3) exhibit genetic stability.

Table AX9-1. Advantages and Disadvantages of Protective Chemicals Used in Assessment of O_3 Effects on Plants

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

Such sensitive plants can be used to detect the presence of a specific air pollutant such as O_3 in the ambient air at a specific location or region and, as a result of the magnitude of their response, provide unique information regarding specific ambient air quality. Bioindicators can be either introduced *sentinels*, such as the widely used tobacco (*Nicotiana tabacum*) variety Bel W3, or *detectors,* which are sensitive native plant species (e.g., milkweed [*Asclepias syriaca*]). The approach is especially useful in areas where O_3 monitors are not operated (Manning et al., 2003). For example, in remote wilderness areas where instrument monitoring is generally not available, the use of bioindicator surveys in conjunction with the use of passive samplers (Krupa et al., 2001) is a particularly useful methodology (Manning et al., 2003). However, the method requires expertise or training in recognizing those signs and symptoms uniquely attributable to exposure to O_3 as well as in their quantitative assessment.

Since the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), many new sensitive species have been identified from controlled exposure studies and verified in the field (Flagler, 1998; Innes et al., 2001). In addition, several new uses of this methodology have been demonstrated, including a national O_3 bioindicator network, studies in wilderness areas, and

mature tree studies. Although it has been difficult to find robust relationships between the foliar injury symptoms caused by O_3 and effects on plant productivity or ecosystem function, visible injury correlations with growth responses have been reported (Table AX9-2) (1998) (2003) (2003). One workshop on the utility of bioindicators of air pollutants led to a useful series of peer-reviewed publications in *Environmental Pollution* (Skelly, 2003).

Table AX9-2. Advantages and Disadvantages of Bioindicators Used to Study O_3 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 symptomotology 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

National network

The U.S. Forest Service in cooperation with other federal and state agencies developed a network of O_3 bioindicators to detect the presence of O_3 in forested systems throughout the United States (Smith et al., 2003). This ongoing program was initiated in 1994; and 33 states currently participate. In a coordinated effort, a systematic grid system is used as the basis of plot

selection, and field crews are trained to evaluate O_3 symptoms on sensitive plant species within the plots (Coulston et al., 2003; Smith et al., 2003).

The network has provided evidence of O_3 concentrations high enough to induce visible symptoms on sensitive vegetation. From repeated observations and measurements made over a number of years, specific patterns of areas experiencing visible O_3 injury symptoms can be identified. Coulston et al. (2003) used information gathered over a 6-year period (1994 to 1999) from the network to identify several species that were sensitive to O_3 over a regional scale including sweetgum *(Liquidambar styraciflua)*, loblolly pine, and black cherry (*Prunus serotina*).

Wilderness areas

The use of bioindicator species as detectors has proven to be an effective technique for deriving a relative estimate of O_3 injury in wilderness areas in both the United States and Europe (Chappelka et al., 1997, 2003; Manning et al., 2002). However, to be truly effective, these regional and national bioindicator studies need the inclusion of air quality data and related growth studies to determine effects on productivity and ecosystem function (Bytnerowicz et al., 2002; Manning et al., 2003; Smith et al., 2003). In addition, O_3 often co-occurs with other air borne pollutants, so it is important to consider that, in some areas, other pollutants may be playing a role as well.

Chappelka et al. (1997, 2003) conducted surveys of foliar injury on several native plant species throughout the Great Smoky Mountains National Park (GRSM), including black cherry (*Prunus serotina*), tall milkweed (*Asclepias exaltata*), cutleaf coneflower (*Rudbeckia laciniata*), and crownbeard (*Verbesina occidentalis*). Visible foliar symptoms were prevalent throughout the Park, indicating that injury-producing O_3 levels were widespread in GRSM.

Manning et al. (2002) recently summarized a multiyear (1993 to 2000) bioindicator project in the Carpathian Mountain range in eastern Europe. They evaluated numerous trees, shrubs, forbs, and vines for possible symptoms of O_3 injury. Observations were made at plots located in the vicinity of either active or passive O_3 monitors (Bytnerowicz et al., 2002). Approximately 30 species of native plant detectors were identified as possible bioindicators, the majority of which (21) were shrubs (Manning et al., 2002). Based on these observations, it was concluded that O_3 concentrations were sufficiently high to impact ecosystems in the region. Similar

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

Mature tree detectors

Many studies have reported visible injury of mature coniferous trees caused by O_3, primarily in the western United States (Arbaugh et al., 1998) and, to a lesser extent, to mature deciduous trees in eastern North America. In an effort to determine the extent and magnitude of visible injury in mature tree canopies, Hildebrand et al. (1996) and Chappelka et al. (1999b) conducted independent studies in the GRSM and the Shenandoah National Park (SHEN). The species examined were sassafras (*Sassafras albidum*), black cherry, and yellow-poplar (*Liriodendron tulipifera*) in GRSM and white ash (*Fraxinus americana*), black cherry, and yellow-poplar in SHEN. Protocols were similar at both parks, and trees were located near O_3 monitors at three different areas in each park. Results from both studies indicated that symptoms of O_3 injury were present in the trees and correlated with O_3 exposure both spatially and temporally. Ozone injury tended to be most severe at the highest elevation, except with yellow-poplar.

Hildebrand et al. (1996) observed significant O_3 exposure-plant response relationships with black cherry. The best relationships were found between foliar injury and the cumulative exposure statistics SUM06 and W126 (see Section AX9.5), indicating that higher O_3 concentrations were important in eliciting a response in black cherry. No O_3 exposure-plant response relationships were found with any species tested in GRSM (Chappelka et al., 1999b); but, when the data were combined for both parks, a significant correlation ($r = 0.72$) with black cherry was found for both SUM06 and W126, and injury was the greatest ($r = 0.87$) at the higher elevations (Chappelka et al., 1999a).

Based on a study in which visible symptoms of O_3 injury were characterized for large, mature yellow-poplar and black cherry trees in GRSM (Chappelka et al., 1999a), Somers et al. (1998) compared radial growth differences among trees classified as sensitive or nonsensitive based on the severity of visible foliar injury observed over a 3-year period (1991 to 1993). Significantly more radial growth was observed over both a 5- and a 10-year period for the nonsensitive compared to the sensitive trees. No significant relationship was found for black cherry tree growth.

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

Because these studies (Somers et al., 1998; Vollenweider et al., 2003a) were not controlled studies and used a small sample of trees, they cannot validly be used to characterize cause and effects related to the visible symptoms and radial growth they describe. However, the results indicate the *possibility* that O_3 is correlated with growth losses in some sensitive genotypes, illustrating the potential usefulness of this visible O_3 injury methodology in assessing effects on the growth rates of mature deciduous trees.

Cultivar comparisons

The idea of using cultivars or isogenic lines of crop species that differed in O_3 sensitivity as sentinels to determine the ambient effects of O_3 in the field was presented in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). The rationale was that comparing the ratio of injury scores or some measure of growth between two different cultivars varying in O_3 sensitivity should be indicative of the relative amount of ambient stress to plants at a given location. A sensitive:resistant ratio close to unity would indicate relatively low O_3 concentrations; and a low ratio, higher O_3 levels. Results from locations differing in O_3 concentrations could be evaluated to develop exposure-response models. The original protocol was derived using two isogenic lines of white clover (*Trifolium repens*) differing in O_3 sensitivity (Heagle et al., 1994b, 1995).

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

(high levels at some sites), and lower O_3 concentrations in Europe were suggested to account in part for the differences between U.S. and European study results. Bermejo et al. (2002), in a study in Spain, improved the model by comparing the biomass ratio of these white clover isolines to measures of O_3 uptake (flux) rather than an exposure index (AOT40). Together, these studies indicate that systems such as the white clover model can help reveal O_3 exposure-response relationships and provide valuable information regarding ambient O_3 conditions in a given location. Table AX9-3 lists the advantages and disadvantages of the use of cultivar comparisons in assessing O_3 effects of plants.

Table AX9-3. Advantages and Disadvantages of Cultivar Comparisons Used in Assessment of O_3 Effects on Plants

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 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 O_3 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

It has been difficult to determine whether O_3 significantly affects tree growth and productivity in the field, because O_3 concentrations are omnipresent and tree response to this pollutant is altered by many factors. The use of dendrochronological techniques to answer questions regarding ambient O_3 effects on forest growth and ecosystem function has recently emerged as a very useful biomonitoring methodology (Cook, 1990; McLaughlin et al., 2002).

The technique is useful when either instrument or passive O_3 monitoring methods are used to determine ambient O_3 conditions.

Initial experiments were primarily correlative in nature and attempted to relate symptoms of visible injury with growth losses as revealed by tree ring analysis (Arbaugh et al., 1998; Benoit et al., 1983; Peterson et al., 1995; Somers et al., 1998; Swank and Vose, 1990). These studies evaluated radial growth patterns determined by cores removed from trees in the presence or absence of overt O_3 injury symptoms.

The method has also been adapted to better understand forest ecosystem function (McLaughlin and Downing, 1995; Bartholomay et al., 1997; McLaughlin et al., 2003). The response of mature loblolly pine growing in eastern Tennessee to ambient O_3 and moisture stress was evaluated by McLaughlin and Downing (1995, 1996). They made radial growth measurements from 12 to 37 times per year using dendrometer bands and determined relationships between O_3, moisture stress, and radial growth. Exposures to O_3 concentrations $\geq$0.04 ppm with high temperatures and low soil moisture resulted in short-term depression in radial growth. Reductions in growth were estimated to vary from 0 to 15% per year and averaged approximately 5% per year.

Bartholomay et al. (1997) examined white pine (*Pinus strobus*) radial growth in eight stands throughout Acadia National Park, Maine over a 10-year period from 1983 to 1992. They related growth rates to several factors, including O_3 concentration. Ozone levels were negatively correlated with radial growth in seven of the eight stands. Site characteristics were important in the relationship: stands growing on shallow, poorly drained soils were most sensitive to O_3 in the late portion of the growing season, possibly due to premature senescence of foliage. However, litterfall measurements were not reported. Trees growing on better sites were more sensitive to O_3 during the entire growing season, indicating the possibility of high O_3 uptake rates throughout the growing season. Although these field studies (Bartholomay et al., 1997, 1996; McLaughlin and Downing, 1995) did not compare the direct effects of O_3 on the two pine species, they indicate that potential interactions exist among O_3 and other climatic and edaphic factors, such as temperature and soil moisture.

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

the vicinity of the trees. They were not able to detect O_3 effects in this 1-year study. Advantages and disadvantages of dendrochronology techniques for evaluating whole-tree physiological responses for individual trees and forest stands are listed in Table AX9-4.

Table AX9-4. Advantages and Disadvantages of Various Dendrochronological Techniques Used in Assessment of O_3 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

The use and evolution of various dendrochronological methods in the field of air pollution effects research is reviewed in detail by McLaughlin et al. (2002). Automated dendrometer bands provide a powerful tool for measuring radial growth responses of trees on an hourly or daily basis. Diurnal patterns of growth can be related to water use and O_3 concentrations using time-series analyses. The major drawbacks of the method are that it is expensive and time consuming.

AX9.1.3.5 Calibrated Passive Monitors

Many studies have used passive monitors in the mapping of ambient O_3 concentrations, especially in remote areas (Cox and Malcolm, 1999; Grosjean et al., 1995; Krupa et al., 2001). Because they are cumulative recording devices, they do not record short-term variations in O_3 concentration but only the total exposure over a given interval, usually between 1 to 4 weeks. Thus, they produce a measurement that resembles the instrumentally derived exposure index SUM00. However, it is common to divide the cumulative exposure by the number of hours of exposure to get an hourly average. In addition, Krupa et al. (2001, 2003) were able to estimate the underlying frequency distribution of hourly O_3 concentrations from passive samplers using models based on a collocated O_3 monitor, showing the potential for passive samplers to provide estimates beyond total O_3 sum.

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

Although based on a single study, the use of passive monitors has potential for assessing crop losses at sites removed from locations with known ambient O_3 concentrations. Provided that the network and calibration sites have similar meteorological conditions, the method yields crop loss estimates that are responses to local ambient O_3 levels as influenced by local meteorological conditions.

AX9.1.4 Numerical/Statistical Methodologies

Proper experimental design strategies including replication, randomization, and experimental protocols are paramount in O_3-effects research. These have been discussed in detail in previous O_3 AQCDs (U.S. Environmental Protection Agency, 1996, 1986), as have the

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

In spite of the rigors of the analyses, many differences occur in the published literature for almost any plant response to O_3 stress. Differences inevitably result from different researchers studying different locations, using different experimental methodologies and genetically different plant material even when using a common species. The techniques of meta-analysis can be used to consolidate and extract a summary of significant responses from a selection of such data.

Despite the differences in responses in the 53 primary studies used, a recent meta-analysis by Morgan et al. (2003) of the effects of O_3 on photosynthesis, growth, and yield of soybean showed "overwhelming evidence for a significant decrease in photosynthesis, dry matter production and yield. . . .across all the reported studies on effects of chronic O_3 treatment." The meta-analysis defined O_3 stress as exposure to ~70 ppb O_3 for at least 7 days and found average shoot biomass and seed yield decreases of 34% and 24%, respectively. Furthermore, although other stress factors such as drought and UV-B did not affect the O_3 responses, elevated CO_2 was found to significantly decrease O_3-induced losses.

The meta-analysis method clearly has the potential to consolidate and refine the quantitative exposure-response models for many species. The majority of the reported growth and physiological responses related to O_3 stress are for individual plants, primarily in various types of exposure chambers. It is difficult to extrapolate these responses to stand/community, ecosystem, or region-wide assessments, particularly in view of the importance of the significant interactions that may occur between plant responses O_3 and other environmental stresses. Along with the shift in effects research to a more ecological approach, these concerns necessitate a move from simple regression analysis to more complex mathematical approaches to handle a wider array of independent input variables than O_3 exposure alone. Other independent input variables that must be accounted for include air and soil temperatures, soil moisture, relative humidity, wind speed, and, particularly in the case of natural systems, biotic factors such as pests and pathogens, plant density/spacing, and measures of plant competition.

Artificial neural network methodology was used by Balls et al. (1995) for "unraveling the complex interactions between microclimate, ozone dose, and ozone injury in clover" and in the study with the protectant chemical EDU, discussed in Section AX9.2.3.3 (Ball et al., 1998). The multi-factor model for predicting the effects of ambient O_3 on white clover developed by Mills et al. (2000) utilized both ANN and multiple linear regression methods.

Models incorporating ANNs are of the "regression" type (Luxmoore, 1988) in contrast to "mechanistic" or "phenomenological" models which have wider applicability. Process-level models of either type have been developed at the organelle, individual plant (Constable and Taylor, 1997; Weinstein et al., 1998), canopy (Amthor et al., 1994), and stand level (Ollinger et al., 1997; Weinstein et al., 2001) and provide estimates of the rate of change of response variables as affected by O_3 over time. However, as pointed out in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), mechanistic process models lack the precision of regression models as well as their ability to estimate the likelihoods of responses. In their extensive reviews, Kickert and Krupa (1991) and Kickert et al. (1999) summarized the advantages and shortcomings of many different models and made the important point that most of the models that have been described provide *consequence* assessments that quantify the magnitudes of effects, but not *risk* assessments that quantify the likelihoods of such effects. Descriptions of several specific models are provided in other sections of this criteria document, and advantages and disadvantages of modeling techniques used in assessing O_3 effects on plants are summarized in Table AX9-5.

Table AX9-5. Advantages and Disadvantages of Modeling Techniques Used in Assessment of O_3 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

AX9.1.5 Improved Methods for Defining Exposure

Ambient air quality is defined in terms of the measured O_3 concentrations in the air at plant height above ground level. Compilations of such concentration data have long been used as surrogates of the exposures to which plants are subjected. However, as long ago as 1965, field research provided evidence that plant response was a function, not of ambient O_3 concentration per se, but of the estimated flux of O_3 to the plant canopy (Mukammal, 1965). Subsequently, Runeckles (1974) introduced the term "effective dose" to define that part of the ambient exposure that was taken up by a plant. Fowler and Cape (1982) later referred it as "pollutant applied dose" (PAD), defined as the product of concentration, time and stomatal (or canopy) conductance, with units g m^{-2}. Such estimates of O_3 uptake or flux provide a more biologically relevant description of exposure than the simple product of concentration and time alone, and they formed the basis of Reich's 1983 "unifying theory" of plant response to O_3 (Reich, 1983).

However, it was not until the early 1990s that the inherent advantages of using O_3 flux rather than O_3 concentration as a basis for determining response effects began to be widely accepted, as demonstrated by the subsequent increase in publications involving flux measurements and modeling (e.g., Fuhrer et al. [1997]; Grünhage and Jäger [2003)]; Grünhage et al. [1993; 1997]; Massman et al. [2000)]; Musselman and Massman [1999]; Pleijel [1998]). A key requirement for flux determination is the measurement of stomatal or canopy conductances, using established porometer/cuvette techniques or eddy correlation methods. The usefulness and relevance of flux as a measure of exposure are discussed in detail in Section AX9.4.

Efforts to develop regional-scale models of O_3 deposition and stomatal uptake are currently under way with a view to providing improved assessments of the risks to vegetation across Europe (Emberson et al., 2000; Simpson et al., 2001, 2003).

AX9.2 SPECIES RESPONSE/MODE-OF-ACTION

AX9.2.1 Introduction

The evaluation of O_3 risk to vegetation requires fundamental understanding of both the functioning of the vegetation and how external environmental influences can alter that function. For biological organisms subjected to atmospheric O_3, those alterations can be complex and

multiple. In addition, biological organisms have plasticity to external interactions due to their complex internal, self-correcting systems, making the task of identifying their "correct" functioning difficult. This section emphasizes reactions of O_3 with the cell and tissue, rather than the whole plant, to describe the fundamental mechanisms known to govern the response of the plant to O_3 exposure.

The many regulatory systems contained in leaves change both as a function of leaf development and in response to various environmental stresses. Leaves function as the major regulators of anatomical and morphological development of the shoot and control the translocation of carbohydrates to the whole plant (Dickson and Isebrands, 1991). This section discusses the movement of O_3 into plant leaves and their biochemical and physiological responses to O_3.

The 1996 criteria document (U.S. Environmental Protection Agency, 1996) assessed the information available at that time concerning the biochemical and physiological responses to the movement of O_3 into plant leaves. This information continues to be valid. Ozone uptake in a plant canopy is a complex process involving adsorption to surfaces (leaves, stems, and soil) and absorption into leaves (Figure AX9-1). However, the initial biochemical changes that result within leaf cells after the entry of O_3 and how these changes interact to produce plant responses remain unclear. The response of vascular plants to O_3 may be viewed as the culmination of a sequence of physical, biochemical, and physiological events. Only the O_3 that diffuses into a plant through the stomata (which exert some control on O_3 uptake) to the active sites within a leaf impairs plant processes or performance. An effect will occur only if sufficient amounts of O_3 reach sensitive cellular sites that are subject to the various physiological and biochemical controls within the leaf cells. Ozone injury will not occur if (1) the rate and amount of O_3 uptake is small enough for the plant to detoxify or metabolize O_3 or its metabolites or (2) the plant is able to repair or compensate for the O_3 impacts (Tingey and Taylor, 1982; U.S. Environmental Protection Agency, 1996). Therefore, a precondition for O_3 to affect plant function is that it must enter the stomata and be absorbed into the water lining the mesophyll cell walls. The response of each plant is determined by the amount of O_3 entering the leaves, which varies from leaf to leaf.

Some potentially significant processes have been investigated since the 1996 criteria document, especially detoxification and compensatory processes. The role of detoxification in

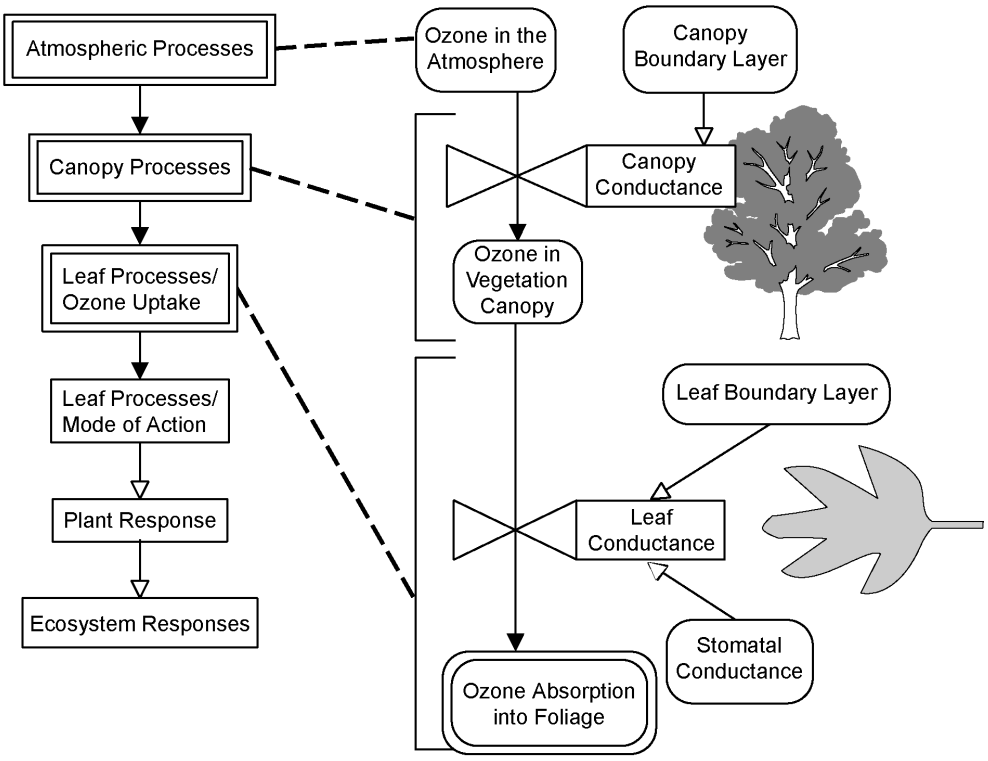


Figure AX9-1. Ozone uptake from the atmosphere. Ozone moves from the atmosphere above the canopy boundary layer into the canopy primarily by turbulent air flow. Canopy conductance, controlled by the complexity of the canopy architecture, is a measure of the ease with which gases move into the canopy. Within the canopy, O_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.

providing a level of resistance to O_3 has been investigated; however, it is still not clear as to what extent detoxification can protect against O_3 injury. Data are needed especially on the potential rates of antioxidant production and on the subcellular localization of the antioxidants. Potential rates of antioxidant production are needed to assess whether they are sufficient to detoxify the O_3 as it enters the cell. The subcellular location(s) is needed to assess whether the antioxidants are in cell wall or plasmalemma locations that permit contact with the O_3 before it has a chance to damage subcellular systems. Although these processes divert resources away from other sinks,

detoxification and compensation processes may counteract the reduction in canopy carbon fixation caused by O_3. The quantitative importance of these processes requires investigation.

As a result of the research since the 1996 criteria document (U.S. Environmental Protection Agency, 1996), the way in which O_3 exposure reduces photosynthesis, especially its effects on the central carboxylating enzyme, Rubisco (ribulose-1,5-bisphosphate/carboxylase), is better understood. The rate of leaf senescence has been shown to increase as a function of increasing O_3 exposure. The mechanism of the increased senescence is not known, and, hence, it deserves further study.

Finally, the role that changes in allocation of resources play in plant response to O_3 is now better understood. Most studies have shown that O_3 decreases allocation of photosynthate to roots. In some cases, allocation to leaf production has increased. Whether these changes are driven entirely by changes in carbohydrate availability or are controlled by other factors (e.g., hormones) is not known. Physiological effects within the leaves inhibit photosynthesis; alter the assimilation of photosynthate and shift its allocation patterns; and can lead to reduced biomass production, growth, and yield (U.S. Environmental Protection Agency, 1986, 1996).

The major problem facing researchers trying to predict long-term O_3 effects on plants is determining how plants integrate the responses to O_3 exposures into the overall naturally occurring responses to environmental stressors. Little is now known about how plant responses to O_3 exposures change with increasing age and size, but this information is crucial to predicting the long-term consequence of O_3 exposure in forested ecosystems.

This section focuses on reactions of O_3 within cells and cellular tissue, in order to explain known mechanisms that govern plant responses. The processes that occur at cell and tissue levels within the leaf will be divided into several steps beginning with O_3 uptake and its initial chemical transformations into a series of currently unknown, but suspected toxic, chemicals (Figure AX9-2). The discussion will then focus sequentially upon various cell regions, their general physiology, and the changes that may occur within a plant after O_3 exposure. This is important because the varying responses of the different plant species in a community ultimately lead to an ecosystem response. Finally, a general summary is presented that discusses the known or suspected changes that occur within the whole plant.

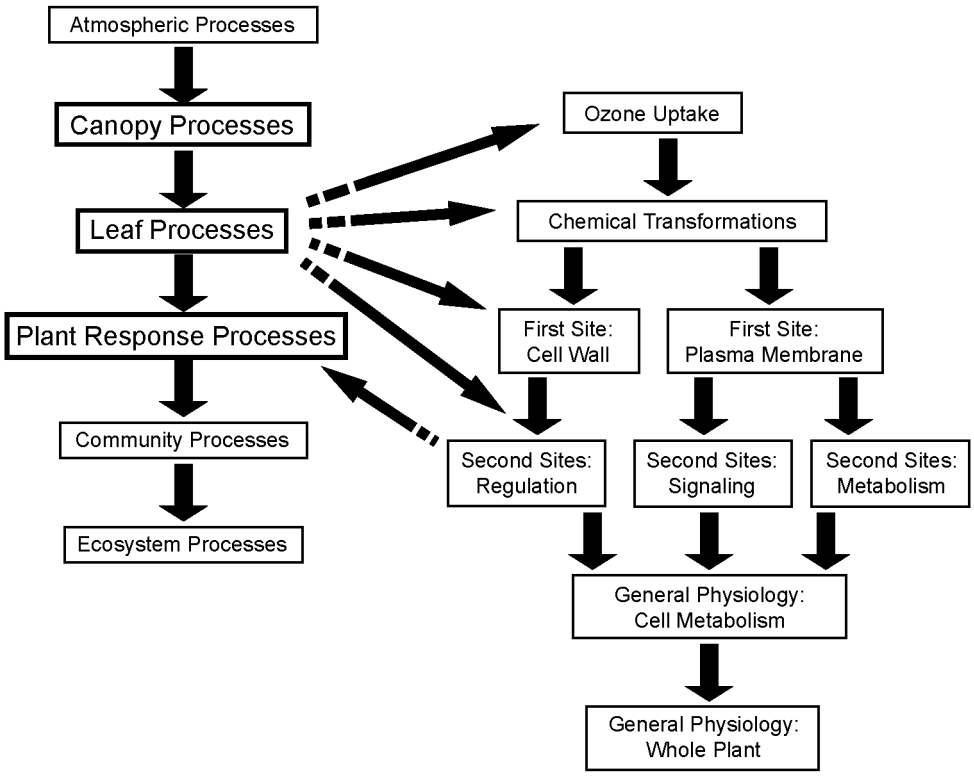


Figure AX9-2. Absorption and transformation of O_3 within the leaf. The varied processes are broken down in to smaller mechanistic steps that lead from uptake of atmospheric O_3 into the alterations which may occur within the individual plant. Each plant responds to the O_3 level and therefore interacts with the total ecological setting to generate an ecosystem response due to the O_3.

AX9.2.2 Mechanisms of Ozone-Induced Plant Alterations

Plants can survive O_3 stress through exclusion or tolerance mechanisms (Levitt, 1972) (Tingey and Taylor, 1982). Ozone may be excluded from tissues or cells via stomatal closure, by extracellular oxidants, or by membrane impermeability to O_3 or its products. Past investigations of O_3 injury have indicated that physiological and metabolic changes occur (Harris and Bailey-Serres, 1994; Heath, 1988; Heath and Taylor, 1997; Reddy et al., 1993). Many of these changes are likely initiated via gene expression. During the last decade, our understanding of the cellular processes within plants has increased. Although the fundamental hypotheses concerning O_3-induced changes in physiology have not changed, a more complete development of the theories is now nearing possibility.

AX9.2.2.1 Changes in Metabolic Processes: Current Theories

The current hypotheses regarding the biochemical response to O_3 exposure revolve about injury and its prevention. These are well discussed by Pell et al. (1997) and are listed below in no order of importance. Although they are listed separately, some may be interlinked and related to each other.

(1) Membrane Dysfunction. The membrane is altered by O_3, principally via protein changes not involving the lipid portions of the membrane (except at extremely high levels of O_3). These alterations involve increased permeability with perhaps lessened selectivity, declines in active transport, and changes in the trigger mechanisms of signal transduction pathways such that the signals are no longer suitable for the state of the cell. The cellular pools and transport systems of $Ca^{2+}/K^{+}/H^{+}$ are the primary suspects.

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

(3) Stress Ethylene Interactions. Visible injury is caused by the interaction of O_3 with stress-induced ethylene, either by direct chemical transformation to a toxic product or by alteration of the biochemical relations at the ethylene binding site.

(4) Impairment of Photosynthesis. A product of O_3 (and less probably, O_3 itself) enters the cell, causing a decline in the mRNA for Rubisco (especially the message RNA species of *rbcS* and *rbcL*) such that Rubisco levels slowly decline within the chloroplast, leading to a lowered rate of CO_2 fixation and productivity. This process is very similar to early senescence and may be linked to general senescence. Alternatively, a false signal is generated at the cell membrane which lowers the transcription of DNA to mRNA. Ozone alters the normal ionic and water relations of guard cells and subsidiary cells, causing the stomata to close and limit CO_2 fixation. In any case, the response of the stomata to the current environment does not promote efficient photosynthesis.

(5) Translocation Disruption. One of the biochemical systems most sensitive to O_3 exposure is the translocation of sugars, such that even a mild exposure inhibits the translocation of carbohydrate (Grantz and Farrar, 1999, 2000).

(6) General Impairment/Disruption of Varied Pathways of Metabolism. This is the oldest and most vague concept of how O_3 alters metabolism. It is based upon early work in which the enzymes and metabolites that could be assayed were. Thus, these results were based upon what could be done, rather than on a coherent hypothesis. The best examples are listed in Dugger and Ting (1970).

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

AX9.2.2.2 Modifications of Plant Physiological Processes

The discussion that follows will focus on physiological processes rather than on species-specific responses; in most cases, the mechanisms of response are similar regardless of the degree of sensitivity of the species. Therefore, *Arabidopsis*, whose physiology and genome continue to be studied and described by a large number of scientists is an appropriate model plant for studying O_3 injury. Though the responses of mature trees and understory plants are critical to understanding plant interactions at an ecosystem level, the time required for trees to reach maturity makes using them to study biological mechanisms an inefficient choice.

The high levels of O_3 used for some investigations do not automatically invalidate the results obtained in those studies. Typically when a new hypothesis is being investigated, extreme levels of the toxicant are used to determine its effects clearly. The older studies that used concentrations as high 1 ppm, an extreme level, helped to define current studies. Later experiments have used concentrations nearer ambient levels. Many of the current studies on physiology use exposures between 0.15 and 0.25 ppm, which though higher than ambient levels in some areas of the country, bypass confounding changes but allow for rapid experiments.

Three forms of air pollutant-induced injury patterns are currently known to exist: (a) acute stress, generated by high atmospheric concentrations of pollutants for short periods of time; (b) chronic stress, generated by lower concentrations of pollutants for long periods of time; and (c) accelerated senescence, generated by very low concentrations of pollutants for very long periods of time. At higher levels, distinct visible injury generally occurs due to cellular and tissue death of regions of leaf mesophyll cells. This leads to a decline in the total area of metabolically active tissue, with consequent loss of membrane integrity, loss of metabolites into

the extracellular tissue space, and formation of oxidative products. When no visible injury is observed, lowered rates of photosynthesis or productivity are often used to document injury. Under these conditions, metabolism is altered and the pool sizes of many metabolites are changed. More importantly, the altered biochemical states within the tissue lead to the inability of the plant to respond properly to existing environmental conditions and to other stressors (Heath, 1988, 1994b; Koziol and Whatley, 1984; Manning and Keane, 1988; Schulte-Hosted et al., 1988).

AX9.2.3 Ozone Uptake by Leaves

Plants respond to O_3 similarly to other stressors on the levels of exclusion, tolerance, and repair (Levitt, 1972). The response mechanism depends upon the O_3 concentration, environmental conditions, and the developmental and metabolic state of the plant (Guzy and Heath, 1993). These responses are detrimental to plant productivity, because they cost the plant metabolic resources. In some cases, the stomata close under the O_3 exposure, excluding the pollutant from the leaf interior and preventing injury. However, if this happens too often, CO_2 fixation is also inhibited and plant productivity suffers.

Atmospheric O_3 does not cause injury, but rather it is the O_3 that enters the plant that causes an effect (Guzy and Heath, 1993; Tingey and Taylor, 1982). Three well-defined, sequential processes control the movement of O_3 from the atmosphere into the sites of action within the leaf and must occur to trigger O_3 stress (Heath, 1980). The processes are (1) entry of O_3 into the leaf, (2) reactions of O_3 and its possible reaction product(s) in the water phase at cell surfaces, and (3) movement of an O_3 reaction product(s) into the cell with enzymatic or chemical transformation of those products in the cell.

Process 1. Entry of O_3 into the leaf. Often incorrectly, the external concentration of O_3 is used to give an indication of "dose" (Heath, 1994a). Ozone-induced changes on a plant's cuticle are minimal, and O_3 does not penetrate the cuticle (Kerstiens and Lendzian, 1989) to cause an effect. As O_3 has no easily measured isotope, virtually no measurements have been done on an actual dose of O_3, i.e., the amount of O_3 which reacts with individual biochemicals in the leaf. Yet the measurement of dose will be the amount of O_3 expected to penetrate into the tissue through the stomata. Dose is expressed as a rate of delivery to a surface area ($mol/m^2\ s^{-1}$).

Whether dose or total accumulation (mole/m^2, rate integrated over exposure time) is most critical for the development of injury remains a major question.

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

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

where the flux, j, into the internal space of a leaf is related to the conductance, g, through the boundary layer and stomata and the gradient of concentration of gas from the outside, C_o, inward, C_i. This formulation has been used for years for both water and CO_2 (Figure AX9-4), and for regions of varied CO_2 concentration that correspond to C_o (CO_2 of the atmospheric air, below the leaf proper) and C_i (CO_2 near the leaf's spongy mesophyll cells) (Ball, 1987; Farquhar and Sharkey, 1982).

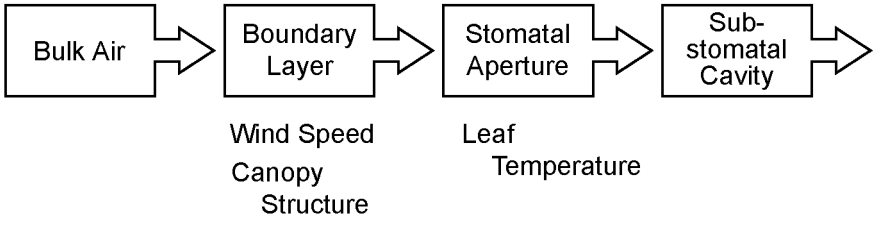


Figure AX9-3. The uptake of O_3 into the leaf. Each of the individual concentration layers of O_3 represents a different process of movement and of plant/microenvironmental interaction. This figure leads into Table 9-6, in which the amounts of O_3 along the pathway are calculated.

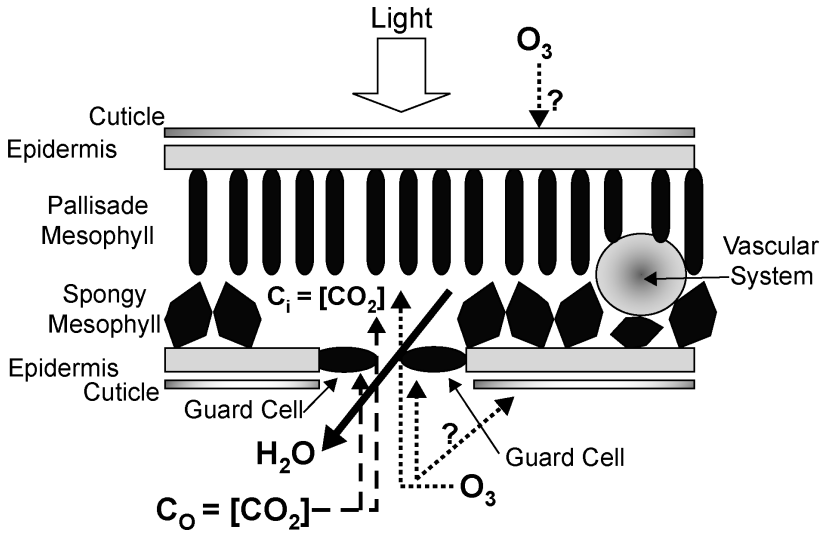


Figure AX9-4. The microarchitecture of a dicot leaf. While details among species vary, the general overview remains the same. Light that drives photosynthesis generally falls upon the upper (adaxial) leaf surface. Carbon dioxide and O_3 enters through the stomata on the lower (abaxial) leaf surface,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

In the past, the internal concentration of O_3 has been assumed to be zero (Laisk et al., 1989), due to early studies that found that virtually no O_3 could pass through a leaf. That was expected because O_3 is extremely reactive with cellular biochemicals**.** If the assumption that the internal concentration zero is correct, then the effective delivery rate for O_3 is given as $g \times C_o$, with stomatal conductance being the major regulatory control (Amiro et al., 1984; Taylor et al., 1982). However, a recent study by Moldau and Bichele (2002) indicated that the internal O_3 concentration may not be zero as previous assumed. Moldau and Bichele (2002) permitted leaves of *Phaseolus vulgaris* L., which have stomata on both upper and lower leaf surfaces, to take up O_3 at a high rate for 3 to 5 min. Exposure of the lower leaf surface resulted in up to 5% of the O_3 that was taken up to be diffused through the leaf, emerging from the stomata on the upper surface. This suggested the presence of above-zero concentrations of O_3 in the intercellular leaf air spaces. The descriptive calculations and plots of Moldau and Bichele (2002) indicate that the rise in internal O_3 level (from both sources of external O_3 concentration) within the first few minutes of exposure is due to its reaction with an antioxidant, most probably absorbate, within the apoplastic space of the leaf (Figure AX9-5). The rate of rise is probably

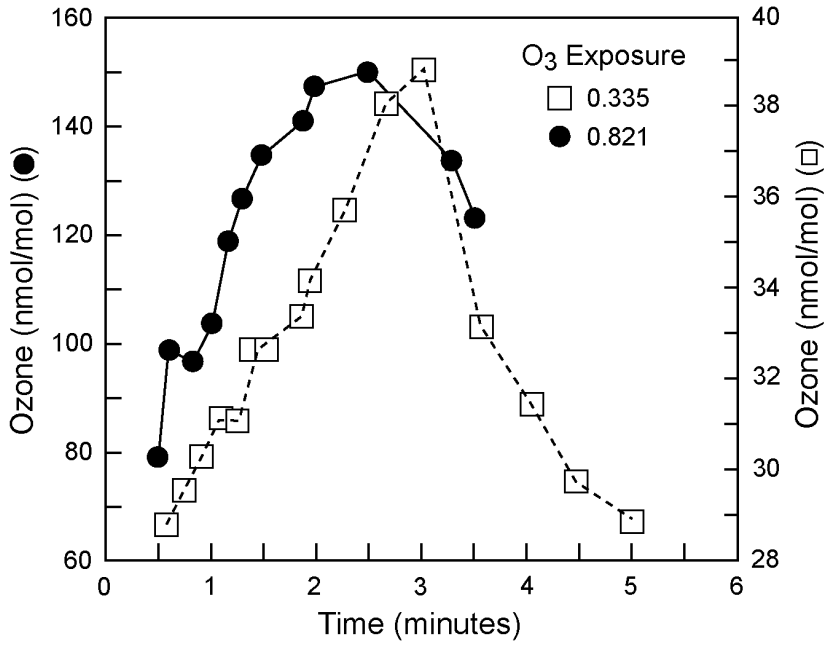


Figure AX9-5. The change in the O_3 concentration inside a leaf with time. Data are from O_3 exposures at two different concentrations.

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

due to more complete penetration of O_3 with a concurrent depletion of the external antioxidant. The rise peaks at about 2 min for 0.82 ppm and 3 min for 0.34 ppm and then falls to a lower level. This may be due to a replenishment of the antioxidant. The authors saw no injury to the plasmalemma (as measured by penetration of a dye) and no change in the stomatal conductance for the lower concentration of O_3 (Moldau and Bichele, 2002). The higher level (0.88 ppm) caused the plasmalemma of the mesophyll cells to pass a dye, and a slight decline in stomatal conductance resulted at about 2.5 minutes. These data suggest that the antioxidant hypothesis is correct.

Gaseous pollutants flow from the substomatal cavity within the leaf through the cell wall into the cell. It is suspected that the internal concentration of the pollutant is not uniform within the cavity (Taylor, and Hanson, 1992). From within the wall, an equilibrium between the gas and aqueous phase must occur at the interface where the gaseous species dissolve into the water according to Henry's Law (Heath, 1980, 1987; Wellburn, 1990). It is important to understand

exactly how much O_3 could move into the tissue of the leaves. Calculations in Table AX9-6 give an indication of the amount of O_3 which may end up near the surface of cells within the leaf. The calculation is done for a standard temperature (25 °C), an ambient concentration of O_3 (0.10 ppm), and for nonspecific leaves. For example, 0.3 ppm would be the same general numbers but multiplied by 3. Similarly for more closed stomata, the value of 1.0 cm/s (equivalent to about 400 $\mu mole^{-2}$-leaf area s^{-1}) for a conductance would be reduced and the smaller values would lead to a smaller amount of O_3 moving into the tissue. Nonetheless, these values give some indication of what sort of chemical concentration can be expected. Under these conditions, a delivery rate of O_3 into the substomatal cavity near the spongy mesophyll tissue of about 0.42 nmol/(L·h) appears to be reasonable.

Process 2. Ozone diffuses into the leaf air spaces and reacts either with varied biochemical compounds that are exposed to the air (path 1) or is solubilized into the water lining the cell wall of the air spaces (path 2). As shown in Figure AX9-6, each reaction has the possibility of transforming O_3 into another chemical species (a toxicant) which, in turn, may react with other chemical species and lead to a cascade of reactions.

Within the stomata, gases react with the water at the cell's surface and generate new species with the components within the cell wall region. The possible varied pathways are depicted in Figure AX9-7. Although these chemical reactions are poorly understood, some of the fundamentals are known (Heath, 1987, 1988; Wellburn, 1990). Ozone reacts with organic molecules at the double bonds to form carbonyl groups and, under certain circumstances, generates peroxides, such as hydrogen peroxides (H_2O_2), superoxide (O_2^-) and its protonated form ($HO_2^\bullet$), hydroxyl radicals ($HO^\bullet$), and peroxy radicals ($HO_2^\bullet$). Other chemicals present in the water phase can lead to many other oxygenated moieties (Figure AX9-6). Each of the steps is generally pH dependent (Jans and Hoigne, 2000; Walcek et al., 1997).

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

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

The level of O_3 in the atmosphere is chosen to be close to a standard and yet make calculations to other amounts easy. The same concept will be used for all standard parameters for these calculations.

DESCRIPTION	VALUES
The atmospheric level of O_3 is given as:	$O_{3\,a} = 0.1$ ppm
For an air temperature of:	$T_a = 25$ °C
The perfect gas law ($pV = nRT$) is used to convert the O_3 level into standard mks. Further, the volume for a mole of gas ($\mathbf{V_o} = 22400$ m^3) will be used, from the perfect gas law with $\mathbf{R} = 8.3144$	
Thus, the concentration of O_3 within the atmosphere is: $$C_{O_3} = \frac{O_{3a} \times 10^{-6} \cdot (T_a + 273.18)}{\mathbf{V_o} \times 273.18}$$	$\mathbf{C_{O_3}} = 4.873 \times 10^{-12}$ 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:	$\mathbf{gs_{wv}} = 1$ cm/s
The amount of O_3 that will penetrate inside the leaf (for a typical concentration of nearly zero inside the leaf), is: $$\mathbf{O_{3_L}} = \sqrt{\frac{18}{48}}\,\frac{\mathbf{gs_{wv}}}{\mathbf{100}}\,\mathbf{C_{O_3}}$$	$\mathbf{O_{3\,L}} = 2.984 \times 10^{-14}$ mol/(m^2·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:	$\mathbf{FW_L} = 30$ mg/cm^2
Thus, the square surface area of the leaf will translate into water space (for concentration of chemicals), as: $$\mathbf{Ar_L} = \frac{\mathbf{FW_L} \times 0.85}{100}$$	$\mathbf{Ar_L} = 0.255$ L/m^2
The maximum amount of toxic compound that will be generated, assuming all the O_3 is converted, is given below. Here the units of the leaf area weight are converted into the mks system and the water space units are converted into L, such that the concentrations calculated will be in mol/(L hr). The final units assume that the O_3 is present (and no back reactions occur) for one hour (short but typical units of exposure). $$\mathbf{O_{3_{Lc}}} = (\mathbf{O_{3_L}} / \mathbf{Ar_L}) \times \mathbf{3600}$$	$O_{3Lc} = 4.21 \times 10^{-10}$ mol/(L·h)
Thus, the maximum amount of toxic chemicals generated per hour in a leaf would be:	$\mathbf{O_{3Lc}} = 0.42$ nmol/(L·h)

Possible errors in these calculations (aside from the input numbers) are (1) the O_3 within the leaf does not react uniformly within the leaf space; (2) the O_3 within the leaf does not totally convert to any one species; (3) varied products of O_3 react, leading to innocuous chemicals; and (4) O_3 reactions can be catalytic and generate more reactions by radical reaction cycling.

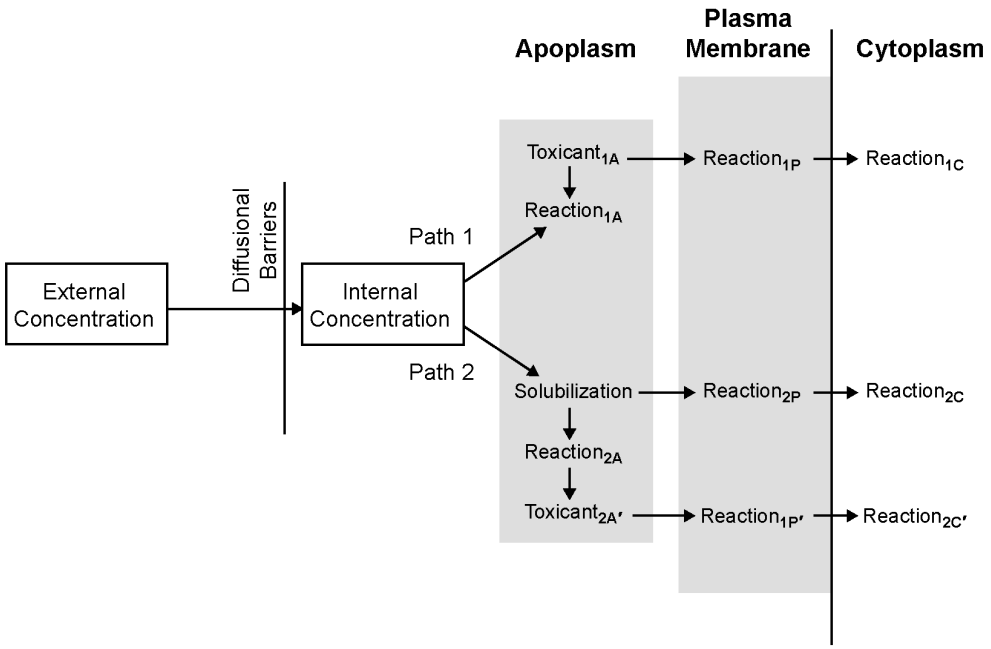


Figure AX9-6. Possible transformations of O_3 within a leaf.

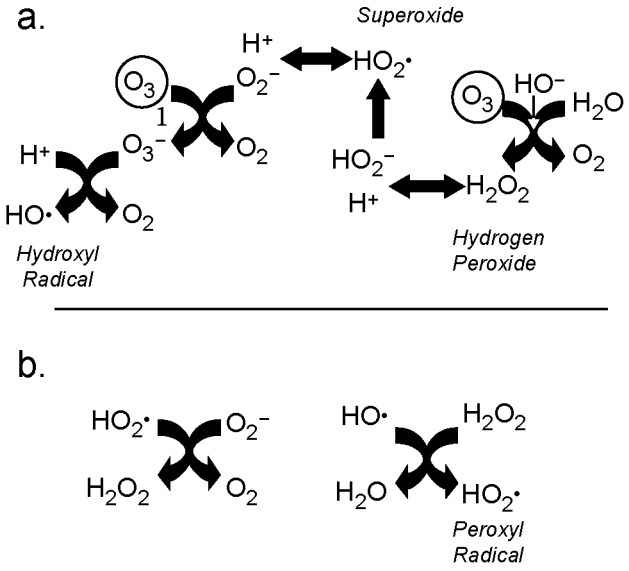


Figure AX9-7. Possible reactions of O_3 within water. (a) Ozone reacts at the double bonds to form carbonyl groups. (b) Under certain circumstances, peroxides are generated.

If the levels are low, it is believed that stimulation of their production is a response to O_3, albeit a slow one (Harris and Bailey-Serres, 1994). Certainly it is possible that chemical modification of wall-specific biochemicals (Castillo et al., 1987) such as glucan synthase (Ordin et al., 1969) and diamine oxidase (Peters et al., 1988) occurs.

Process 3. *Movement of reaction product(s) into and enzymatic or chemical transformations within the cell*. It is believed that the initial site of O_3 injury is near or within the plasma membrane. Certainly, membrane functions, such as membrane fluidity (Pauls and Thompson, 1980), permeability (Elkiey and Ormrod, 1979), K^+-exchange via ATPase reactions (Dominy and Heath, 1985), and Ca^{2+} exclusion (Castillo and Heath, 1990), are changed. The similarity of wounding responses (Langebartels et al., 1991) and O_3-induced membrane disruption suggests the induction of normal wound-regulated genes (Mehlhorn et al., 1991; Sandermann, 1998). This implies that O_3 can react with cell-wall components that are connected to the cytoplasm through the cell wall and membrane by membrane-specific proteins that are not directly involved with transport.

Ozone is soluble in water and once having entered the aqueous phase, it can be rapidly altered to form oxidative products that can diffuse more readily into and through the cell and react with many biochemicals. Again, the presence of an internal antioxidant would be critical to reduce the concentration of most oxidants. A toxic product of O_3 may migrate through the cytoplast to react with photosynthetic processes, or a spurious signal generated at the membrane may affect some control process or signal transduction pathway (Schraudner et al., 1998; Overmyer et al., 2000, 2003; DeCaria et al., 2000; Rao et al., 2002; Booker et al., 2004; Leitao et al., 2003; Rao and Davis, 2001; Sandermann, 2000; Vahala et al., 2003b).

AX9.2.3.1 Possible Reactions Within the Leaf

Ozone can react with many compounds within the substomatal cavity of the leaf[1] to produce a variety of oxidizing and toxic chemicals. Some of the possible reactions that will generate H_2O_2, $HO^{\bullet}$, and SO_2^-, as well as charged O_3 intermediates, are indicated in Figure AX9-8. Many of these complex reactions have been studied within water solutions through

[1]The volume of the substomatal cavity (that are within the leaf immediately below the stomata) must be regarded as the region in which most O_3 reactions occur. That volume, at a relative humidity of near 100%, possesses many diverse surfaces with varied bonding, which could alter the fate of O_3.

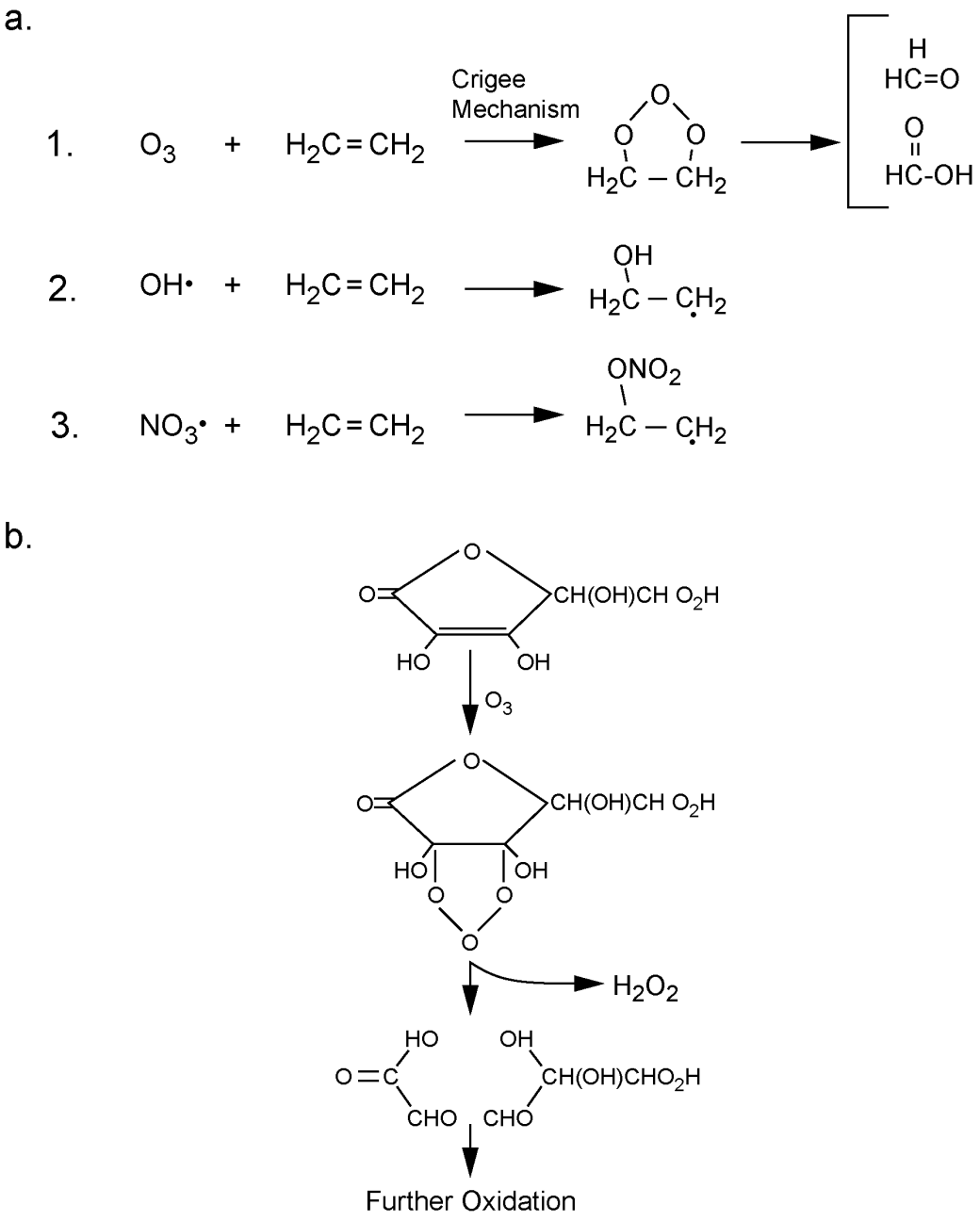


Figure AX9-8a,b. The Crigee mechanism of O_3 attack of a double bond. (a) The typical Crigee mechanism is shown in which several reactions paths from the initial product is shown. (b) Typical reaction of ascorbic acid with O_3.

Source: Adapted from Mudd (1996).

research of O_3-induced water purification and are very dependent upon solutes present with the solutions, including H^+ (see Von Gunten [2003]). An important point is that in alkaline media, O_3 forms H_2O_2, but in acid media, O_3 is relatively stable in the absence of free metal ions.

The rates of reaction of O_3 with several important compounds, including those with a double bond, the so-called Crigee Mechanism shown in Figure AX9-8, can be calculated from the reaction coefficient as given by Atkinson (1990) (Table AX9-7). The double bond of the ascorbate molecule is particularly sensitive to O_3 attack. Because of the ring formation of the ascorbate molecule, an unstable ozonide product is formed, which then accelerates the breakage of the double bond, leading to the formation of two products. These products are relatively unstable and can lead to further reactions not shown in Figure AX9-8. The rates of reactions can be calculated (Heath, 1987). At a local concentration of 25 μM O_3, it would take 5000 s (83 min) for all of the O_3 to react if there was no further flow of O_3. Clearly, O_3 does not react rapidly with the compounds in Table AX9-7 and, although some of the products would be formed through the Crigee Mechanism (see Figure AX9-8a), they would be low in concentration[2]. While other radicals, such as the hydroxyl radical (see Figure AX9-8b) can attack double bonds, the products differ. Of particular note for later discussion, is the reaction of O_3 with ascorbate (see Mudd (1996) (see Figure AX9-8b), which will cleave the double bond in the ring. Unfortunately, little work has been done to characterize possible products within the leaf (but see next section).

In a paper discussing the stability and reactivity of O_3 in the pulmonary air/tissue boundary, Pryor (1992) calculated that O_3 has a half-life of about 7×10^{-8} s in a bilayer. However, the transit time through the lung lining fluid layer is about 2×10^{-6} s, based upon a reasonable estimate for the diffusion of O_3. This means that O_3 would suffer nearly 29 half-lives[3] in passage through the layer, reducing it to about 3×10^{-9} of the original concentration — zero for all practical aspects. In the same publication, Pryor points out that any sulfhydryl or ascorbate would interact strongly with O_3, further reducing its net concentration. The reactivity of cysteine is 10^9, while the reactivity of tryptophan, methionine, polyunsaturated fatty acids,

[2]For example, hydroxylmethyl hydroperoxide would be expected to be formed by the reaction of O_3 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.

[3]Here a half-life is the time that it takes the reactive species to travel a distance in which it loses 50% of its initial concentration. Therefore for a 29 half-life, the concentration has been reduced by 2^{-29} or about a 10^{-9} decline.

Table AX9-7. Some Rates of Reaction of Ozone With Critical Biochemicals

[a] Double bond reactions. The second column is taken from Atkinson (1990) and transformed into Column 3. Those rate coefficients are used to calculate the rate of reaction at a concentration of 10 ppm for the organic and 0.1 ppm for O_3 in the air stream within the leaf (localized concentration of about 25 mM, see Table AX9-6).

Compound	$\times 10^{-18}$ cm^3/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) $O_3 + OH^- + H_2O \rightarrow H_2O_2 + O_2 + OH^-$	$k_1 = 3.67 \times 10$ mole^{-1} L s^{-1}
(2) $O_3 + O_2^- \rightarrow HO^- + 2\ O_2 + OH^-$	$k_2 = 1.26 \times 10^9$ mole^{-1} L s^{-1}
(3) $O_3 + HO_2^- \rightarrow HO^- + O_2^- + O_2$	$k_3 = 2.09 \times 10^6$ mole^{-1} L s^{-1}

[c] Possible Concentrations of Other Oxidative Species. Table from Heath (1987). Based upon 100 ppm O_3 in gas stream.

	Concentration (M)			
Species	**pH 7**	**pH 9**	**Molecules within wall**	
Superoxide Radical ($O_2^{\bullet}$)	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 ($HO_3^{\bullet}$)	1.48×10^{-16}	1×10^{-18}		

Number of molecules within apoplastic space of (10^{-12} L) at 0.1 ppm O_3.

and tyrosine is about 2×10^6 and that of phenylalanine is only 10^3. These numbers are similar to what has been found for O_3 reactivity with amino acids and proteins in aqueous solutions. In glycophorin (Banerjee and Mudd, 1992) and cytochrome C (Mudd et al., 1997b; Mudd et al., 1996) in aqueous solutions, only the methionine was oxidized by O_3, producing sulfoxide. In other proteins lacking methionine, tryptophans were oxidized only if they were in an exposed position on the surface of the proteins (Mudd et al., 1997b). Treatment of red blood cell ghosts

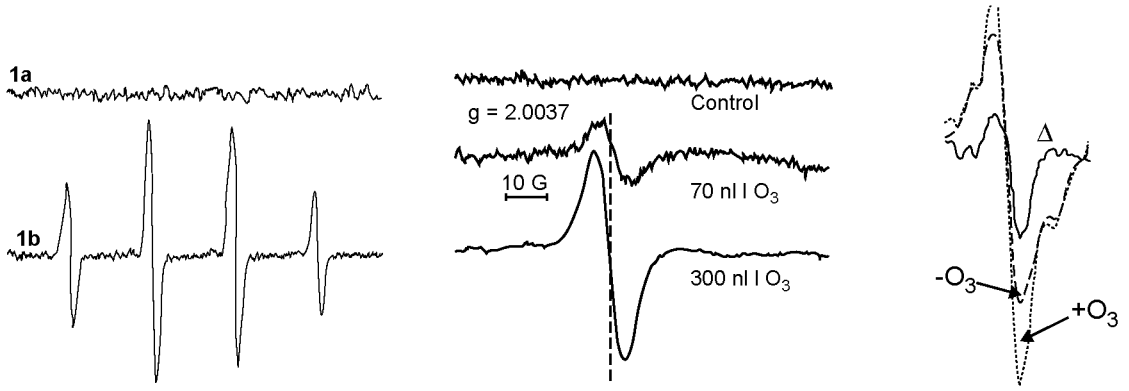
with O_3, oxidized peripheral proteins of the plasma membrane before it oxidized lipids (Mudd et al., 1997a).

AX9.2.3.2 Toxicants Within the Wall Space

While Mehlhorn et al. (1990) are often thought to have shown that free radicals were formed in plant leaves under O_3 exposure, careful reading of that paper clearly shows that there was no real evidence of free radicals induced by O_3. Living tissues have many free radical signals, making it difficult to observe changes in free radicals. Further, the work of Grimes et al. (1983) has also been cited as showing the presence of free radicals in living tissues due to O_3 exposure; however, no radical signals were found unless certain organic acids (e.g., caffeic acid) were added to the tissue with the O_3 exposure. They used the radical trap TMPO (tetramethylphrrolise 1-oxide) which reacts with many types of free radicals to form a stable radical that can be used to "trap" or increase the amount of radical present (see Figure AX9-9a). Ozone would directly react with this trap only if it were bubbled into the solution, not passed over the top of the solution. In the presence of sorbitol or caffeic acid, the trap would indicate the presence of OH radical, which would mean that $O_3 \rightarrow HO^{\bullet}$. Superoxide dismutase, catalase, or EDU had no effect upon this signal, suggesting O_2^- and H_2O_2 were not involved in the above sequence. Both O_3 and O_3 plus caffeic acid had no effect upon the protoplasts' intactness or viability. Thus, 10^{-5} M $HO^{\bullet}$ and/or 0.30 to 0.40 ppm O_3 did not react with the cell membrane. They found no signal in normal cells after subjecting the leaf to O_3 and concluded that the radicals were produced via a concerted mechanism with the acid. This does not fit with the mechanism postulated by Mehlhorn et al. (1991), which involved a reaction of wound-induced ethylene and O_3 at the wall level to generate some free radicals.

The hypothesis that the production of wound-induced ethylene by O_3 exposure and its reaction with O_3 would result in the production of radicals was tested by Mehlhorn et al. (1990), using electron paramagnetic resonance spectroscopy. After 4 h of 300 ppb, an EPR signal of a compound was detected which resembled a butonyl radical (Figure AX9-9b [Mehlhorn et al., 1990]). Using 70 ppb, the signal was reduced by about one third that of an ethyl radical[4],

[4]The reaction would be: $O_3 + H_2C = CH_2 \rightarrow$ varied C-1 compounds, due to double bond cleavage, at a rate constant of 1.7×10^{-18} cm^3/molecule sec = 1.02×10^3 M^{-1} s^{-1} (Atkinson, 1990). This should be compared with a reaction of the hydroxyl radical with ethylene, which has a rate constant of 8.52×10^{-12} cm^3 $molecule^{-1}$ s^{-1}, or $10^6 \times$ faster.



a.

ESR spectra from solution treated with O_3 at 0.3 µg/g dissolved for 60 s (1a) control, (1b) with caffeic acid; spin trapped: DMPO (Grimes et al., 1983)

b.

ESR spectra from bean (*Phaseolus vulgaris* cv. Pinto) leaves pretreated with spin trapped: PBN after a 4-h fumigation with O_3 (0-300 nl/L) (Mehlhorn et al., 1990).

c.

EPR signal in white light before (broken line) and after (dotted line) exposure of a bluegrass leaf to 1000 µg m^{-3} O_3 in the EPR cavity for 1h. The solid line depicts the difference signal. (Runeckles and Vaartnou, 1997)

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 µg 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.

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

Runeckles and Vaartnou (1997) (Figure AX9-9c) discovered a signal by subtracting other EPR signals of the leaf, which seemed to be due to an O_3 reaction with plant material, using 0.48 ppm O_3. This difference signal looked very much like O_2^-. At a lower concentration, they observed that this signal still occurred but accumulated more slowly. Both bluegrass and ryegrass leaves seemed to saturate after about 5 h of exposure at 22 to 28 units of signal, while radish leaves reached a maximum of 7 units at 3 h and then declined. The problem, which is

typical of any of these methods, was that the detached leaf had to be rolled and placed into the EPR detection cavity. Reichenauer et al. (1998) also detected an undefined free radical signal that seemed to be related to a Mn(II) spectrum. The Nandu and Perlo cultivars of wheat were more sensitive to O_3 than Extradur (according to growth rate and closure of stomata under an O_3 exposure of 80 ppb for 8 h/day, 7 days/week over 100 days), and these more sensitive cultivars had a greater, but insignificant (P = 15%), EPR signal. Thus, data showing any production of a free radical must be approached with some skepticism.

With an O_3 delivery rate of about 25 μM/h (Table AX9-6), only 250 μM would be found after a full day, if all of the O_3 were stable. While the use of free radical traps is the best method available to observe any build-up of radicals, the traps are not as specific to individual radicals. Currently, studies should be looking for hydroxyl radicals, superoxide, hyroperoxides, ethylene radicals, and ascorbate radicals.

AX9.2.3.3 Products of Ozone

Ozone should reach a certain concentration in the substomatal cavity, which is dependent upon its entry speed and its reactivity with the wall constituents. Once near the apoplastic space, O_3 moves in two different pathways (Figure AX9-6). It can react with constituents that are within the wall as a gas in reaction 1A (path 1); or it can solubilize into a water space and travel to another region within the water space and react through reaction 2A (path 2).

Hydrogen Peroxide

Hydrogen peroxide, until recently was thought to be purely a toxic compound for cells. However, it is now clear that it functions as a signaling molecule in plants and mediates responses to abiotic and biotic stressors (Figure AX9-10). Generation of H_2O_2 is increased in response to various stressors, implicating it as a key factor mediating the phenomena of acclimation and cross tolerance, in which exposure to one stressor can induce tolerance of subsequent exposure to the same or different stresses (Neill et al., 2002). The signaling response to attack by invading pathogens using H_2O_2 has been described (Mehdy, 1994; Simon-Plas et al., 1997). The reactions leading to hypersensitive cell death are caused by a pathogen recognition step (Figure AX9-10a), probably due to the plant cell wall releasing oligosaccharides in response to the pathogen enzymatically breaking down the cell wall to penetrate it. A feed-forward

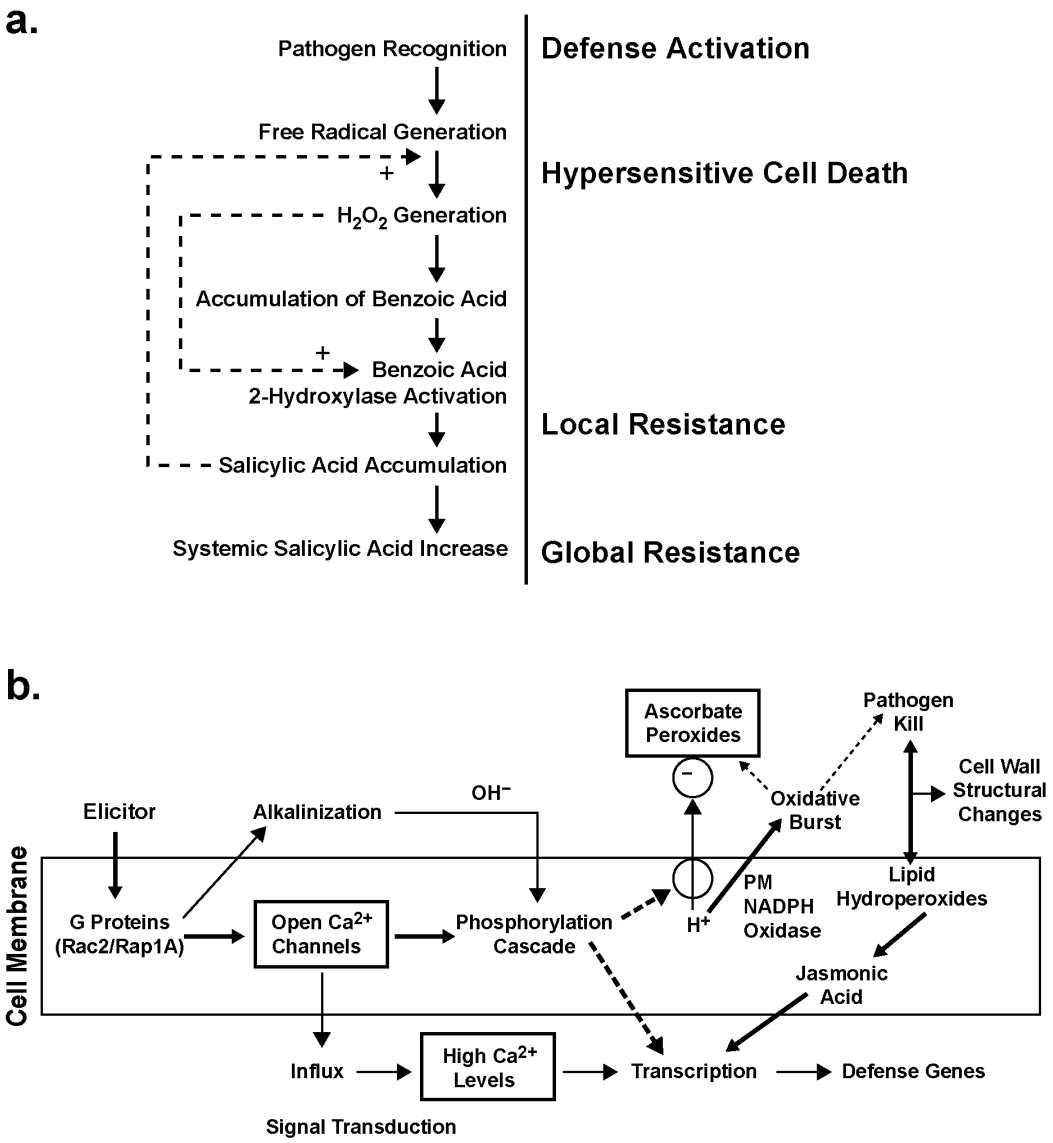


Figure AX9-10. Pathogen-Induced Hypersensitivity. (a) The reactions leading to hypersensitive cell death and the formation of a global response of salicylic acid. (b) The cascade of the elicitor-induced reactions within the cell.

step in which H_2O_2 increases the level of benzoic acid leads to the activation of the hydroxylase step in the production of salicylic acid and to a feedback step in which the salicylic acid increases the production of H_2O_2 (Leon et al., 1995).

An elicitor, e.g., a bacterial or fungal pathogen, induces a cascade of reactions within a cell (Figure AX9-10b). Some of the lipid reactions are thought to be due to the opening of the Ca^{2+} channels and the alkalination of the cell wall region. The oxidative burst due to H_2O_2 production is believed to lead to the transformation of a small population of lipids into jasmonic acid, which is a secondary messenger.

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

It is believed that the first species generated through a one-electron reduction of molecular oxygen is SO_2^-. That generation is carried out using a cytochrome b_6 by the NAD(P)H oxidase located on the cell membrane (Auh and Murphy, 1995). In the acid region of the cell wall, SO_2^- is converted by a protonation and dismutation to H_2O_2. The induced oxidative burst is believed to play a role in stimulating the Cl^- and K^+ efflux, generating an alkalinization of the extracellular space (Cazale et al., 1998). In the wall region, H_2O_2 is not especially toxic, as no necrosis was reported in tobacco when 500 mM peroxide was infiltrated into the leaf tissue. However, the production of salicylic acid and benzoic 2-hydroxylase can be induced with only 30 and 0.3 mM H_2O_2, respectively, indicating some metabolic signaling (Leon et al., 1995). On the other hand, 1M H_2O_2 infiltrated into soybean will generate lipid peroxidation after 1 h with a peroxidation rate of 15 nmol/g-FW·h (Degousee et al., 1994). Cells react to the system[5] and generate peroxide scavenging compounds within 1.5 to 2 hours, which appear to "mop up" the excess H_2O_2 (Baker et al., 1995).

After O_3 exposure in birch, H_2O_2 has been found in the wall (Pellinen et al., 1999). By using $CeCl_2$ as a cellular stain for H_2O_2 (as a cerium perhydroxide precipitate), Liu et al. (1995) observed a gradual development of stain after 8 h of O_3 exposure (at 150 ppb). After 2 h exposure, H_2O_2 stain was visible on the surfaces of both sets of mesophyll cells. Accumulation of H_2O_2 stain continued for 16 h after exposure, suggesting a triggered-reaction rather than O_3

[5]Soybean 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.

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

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

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

Certain levels of ABA within the leaf lead to stomatal closure. The inactivation of a phospho-tyrosine-specific protein phosphatase (ABI2) is an inhibitor of stomatal opening induced by ABA but that enzyme is inhibited by H_2O_2 (Meinhard et al., 2002). This means that H_2O_2 shifts the sensitivity of the stomatal opening to ABA (Figure AX9-11), making the stomatal complex more sensitive to ABA. Thus, for a given level of ABA present in the guard cell complex due to environmental factors (e.g., low humidity, high air temperature, or low soil water potential), the generation of H_2O_2 would (by inhibiting ABI2) induce a closure of the stomata by increasing the sensitivity of the guard cells to ABA. In the past, it has been difficult to understand why O_3 would often decrease conductance in some cases, but not always (Heath, 1994b). This interaction between H_2O_2 and ABA could help understand this complexity.

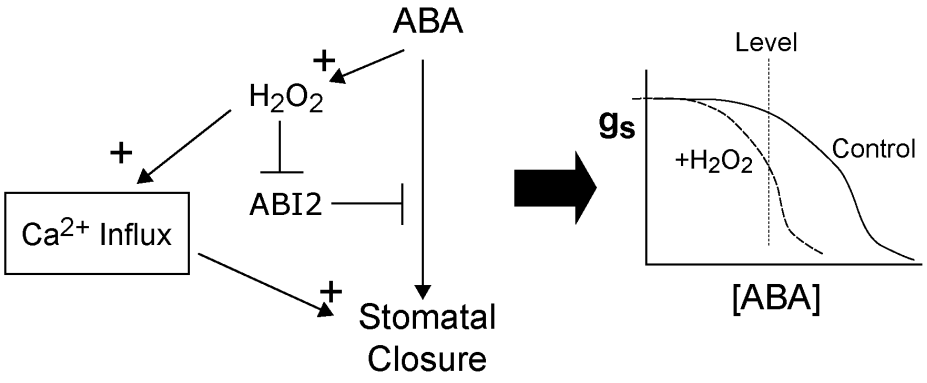


Figure AX9-11. The interaction of H_2O_2 and Ca^{2+} movements with ABA-induced stomatal closure. It is well known that certain levels of ABA within the leaf lead to closure of the stomata within the leaf. That level, however, can be shifted to make closure more or less sensitive to a given level of ABA. Recently it has been shown that H_2O_2 (externally or produced by the plant) within the cell wall region can shift that sensitivity. Here ABA stimulates the production of H_2O_2, which in turn increases the rate of Ca^{2+} moving from the wall region into the cytoplasm. That shift in internal Ca^{2+} level increases the closure of the stomata. Hydrogen peroxide also blocks the activation of a polypeptide (ABI2) that inhibits stomatal closure seemingly induced by ABA.

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

Ethylene Reactivity

Ethylene (ET) is produced when plants are subjected to biotic stressors, e.g., attacks by insects, fungi, and bacteria or abiotic stressors such as wounding or environmental stressors such as heat, cold, or oxidative stress and O_3. If an O_3 stress has induced a wounding response with ET release, then ET within the substomatal cavity could react with O_3, generating some relatively noxious chemicals (see Figure AX9-6). The relationship between O_3 injury and wounding is supported by the observation by Mehlhorn et al. (1991) that an inhibitor of ET formation, AVG (an inhibitor of ACC synthase, a committed step to ET production), would block ET formation and inhibit visible injury. Other studies with polyamine (which is closely linked to ET production), including those of Ormrod and Beckerson (1986) who fed polyamines to the transpirational stream and prevented visible injury, suggested a close involvement of both

pathways to the production of visible injury. Both the lack of ET production and an increased level of polyamines slowed or prevented visible injury.

This concept was taken another step by Langerbartles (1991). The linkage to the pathogen wound responses and visible injury is well established (Sandermann, 1996). Sandermann (1998) used a system of Bell B and W3 tobacco, plants with differential O_3 sensitivities, in which the O_3 exposure level was chosen such that the sensitive cultivar was injured, while the tolerant one was not. This led to a marvelous control that could be used to their advantage. They followed a time sequence to show that the rise of varied systems followed the same order as seen for a pathogen attack (Heath, 1994a).

More recent studies, however, indicate that O_3 responses resemble components of the hypersensitive response (HR) observed in incompatible plant-pathogen interactions (Sandermann, 1998). The similarity to the HR response may be related to the occurrence of ROS in the apoplast. The O_3-derived ROS apparently trigger an oxidative burst in the affected cells by an as yet unknown mechanism. An oxidative burst is similar to one of the earliest responses of plants to microbial pathogens and is an integral component in HR-related cell death (Overmyer et al., 2000).

In plants exposed to O_3, ET synthesis is a result of the specific ET induction of the genes encoding 1-aminocyclopropane-1-carboxylase synthase (ACS), one of the fastest and most obvious responses to O_3, which has been mechanistically linked to the regulation of O_3 lesion formation. Biosynthesis of ET inhibited, with ACS inhibitors significantly reduced, the induction of lesion formation in plant leaves exposed to O_3 (Mehlhorn et al., 1991; Mehlhorn and Wellburn, 1987; Vahala et al., 2003b). Ethylene biosynthesis correlates best with O_3 exposure (Overmyer et al., 2000; Vahala et al., 2003b). These data support the concept that elimination of ET formation will prevent visible injury.

Ethylene-Interaction with Injury and Conductance

Increased ET production by plants exposed to O_3 stress was identified as a consistent marker for O_3 exposure decades ago (Tingey et al., 1976a). They exposed more than 20 plant species and cultivars to O_3 to determine whether the production of O_3-induced stress-ET could be used to determine differences in plant sensitivity to O_3. Their studies suggested that increased

production of stress-ET correlated well with the degree of foliar injury that developed within hours or days after O_3 exposure. The amount of ET released was exponentially related to the O_3 exposure. Furthermore, the amount of O_3-induced ET declined with repeated exposure, indicating an acclimatization to O_3. This acclimatization effect associated with repeated wounding has not yet been well described. The release of wound-induced ET is not linear with time, but declines after the initial response (Stan et al., 1981), as is also seen after O_3 exposure (Stan and Schicker, 1982). The stress-induced ET production correlates better with O_3 exposure level than with exposure duration. In other words, peaks of high O_3 (rather than accumulated dose) generate a higher rate of ET release, at least for a single O_3 exposure under an acute dose.

The production of ET after an O_3 exposure is thought to be a typical wounding response (Tingey et al., 1975). Prevention of ET release may prevent the formation of visible injury (Mehlhorn and Wellburn, 1987). However, the question arises as to whether this effect was limited to the prevention of visible injury or if the chemicals used to prevent ET release closed the stomata. Using *Glycine max* L., Taylor et al. (1988b) showed clearly that AVG did not necessarily close stomata nor inhibit carbon assimilation per se.

The correlation of ET release with O_3-induced visible injury was likewise shown in pea cultivars (Dijak and Ormrod, 1982). With O_3 exposure (generally 6 h at 0.3 ppm), the stomata closed by ~50% within 3 h after a dose of 3×10^{-5} mol cm^{-2} (with an average rate of 2×10^{-9} moles cm^{-2} s^{-1}, as calculated from their data). Both sensitive and insensitive cultivars had a visible-linked-injury ET release, but sensitive cultivars scored higher both in visible injury and in ET release after a given exposure.

Gunderson and Taylor (1988, 1991) used exogenous ET to alter the gas exchange of *Glycine max* and found an exponential, but not simultaneous, decline of both stomatal conductance and carbon assimilation with ET. Interestingly, the exogenous ET caused a slight rise in difference of CO_2 within and without the leaf, indicating a lowering of internal CO_2, which was not observed in the experiments of Farage et al. (1991) for O_3 exposure. Ethylene inhibits both stomatal conductance and carbon assimilation to some extent (Taylor et al., 1988b). Thus, one could postulate that O_3 generates a wounding response with a production of ET, which would, in turn, generate the change in stomatal conductance and photosynthesis. Clearly, these multiple events may have confounded some earlier studies.

AX9.2.3.4 Antioxidants Within the Apoplastic Space

The first line of defense against O_3 is a closure of the stomata to exclude its uptake. This is counterproductive for efficient photosynthesis, but some amount of closure limits the rate of O_3 deposition into the leaf tissue to allow for a secondary line of defense to detoxify the O_3. The secondary line of defense involves a range of antioxidants, which are highly reactive to the types of chemicals that can be generated by O_3. Several antioxidant proteins are stimulated by O_3 in *Plumbagini folia*, including glutathione peroxidase (GSH-P_x), SOD, and catalase. The timescales for changes in their levels vary: some rise rapidly, while others rise more slowly. The pattern of changes in these particular proteins varies greatly among different species and conditions.

Ascorbate Within the Cell Wall

Most of the recent reports indicate that ascorbate within the cell wall is the real first line of all defense. Ascorbate within the wall declines when the tissue is exposed to O_3 (Luwe et al., 1993; Moldau, 1998). This decline appears to be closely linked to the amount of O_3 penetrating the leaf tissue.

It has long been suspected that intracellular antioxidants play a role in preventing O_3-induced injury to plant cells. Variation in the types of biochemical compounds present in the apoplastic space can give rise to a multiplicity of reactions with O_3, but the predominant biochemical species is ascorbate. Ascorbate is water soluble, present in the solution where O_3 can dissolve, and is highly reactive. Unfortunately, a variety of antioxidants are found throughout the cell and any measurements of one particular type within the total leaf tissue can give misleading results. For example, ascorbate is present within the cell wall, cytoplasm, and chloroplasts (Burkey, 1999; Moldau, 1998); and ascorbate can move between the cytoplasm and the cell wall with relative ease (Figure AX9-12) (Bichele et al., 2000). The total of all ascorbate pools is measured when the tissue is ground and assayed. If the cell wall ascorbate concentration drops by 50% due to O_3 exposure but all other tissue concentrations remain the same, the measurement of the total loss is dependent upon the amount of ascorbate within the cell wall. Turcsányi et al. (2000) showed that, compared to the concentration of apoplastic ascorbate, the rest of the cells contained about 38 times as much. So a 50% loss of apoplastic ascorbate would be converted into only 2 to 3% loss of the total ascorbate.

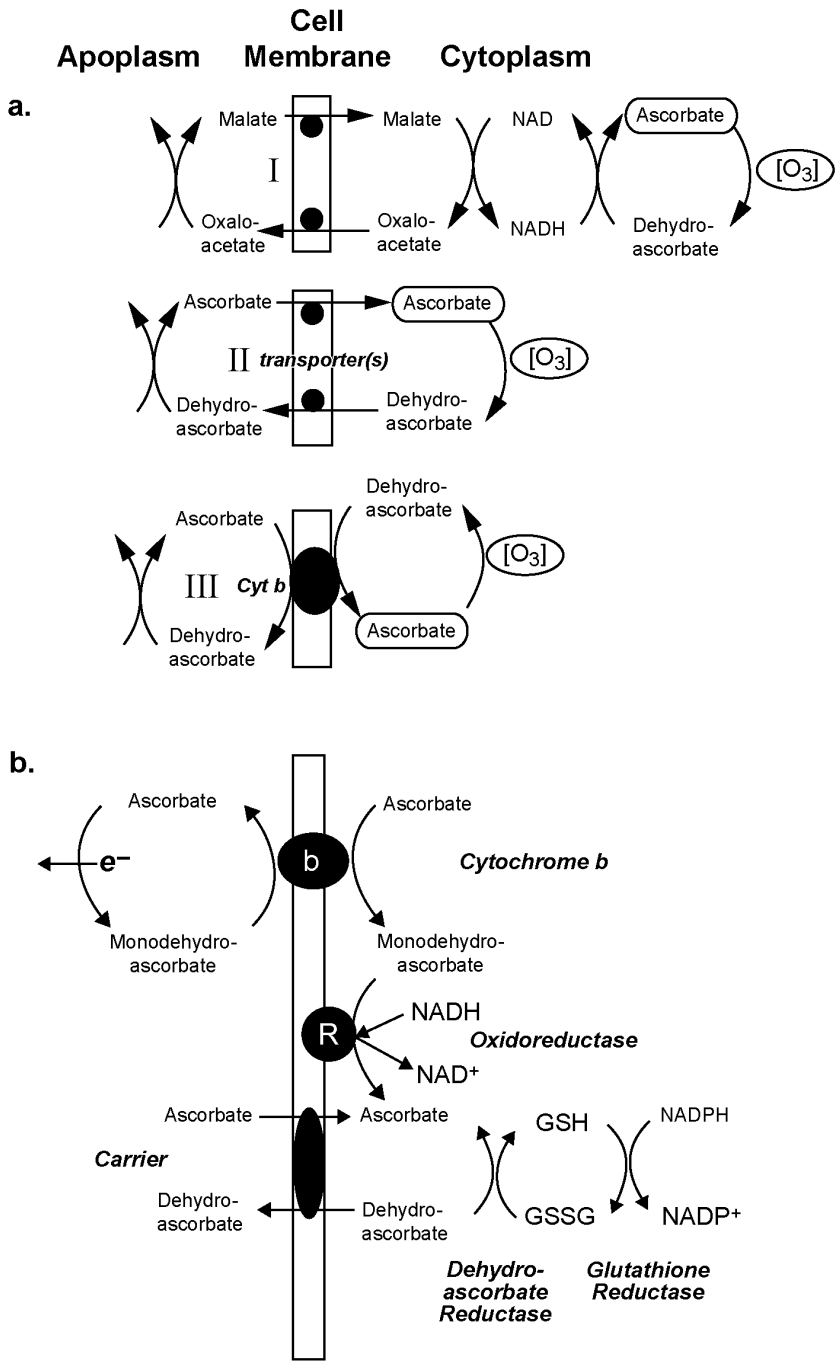


Figure AX9-12. The reaction of ascorbate within the apoplasm of the cell wall and its ultimate reduction/oxidations.

(a) Movements of reducing power (from Dietz [(1997]).

(b) The use of glutathione to maintain the level of ascorbate within the cell wall region (from Horemans et al. [2000]).

The ascorbate deficient *Arabidopsis thaliana* mutant has proven to be a powerful tool in furthering the understanding of ascorbate biosynthesis in plants (Smirnoff et al., 2001). Three classes of mutants were formed when *A. thaliana* seed was mutagenized with ethyl methanesulfonate: (a) those deficient in SOD, (b) those that failed to accumulate more antioxidant proteins upon increased O_3 exposure, and (c) those that were deficient (but not depleted) in ascorbate. The low-ascorbate mutant type had 50 to 60% less ascorbate than the wild type and displayed more foliar injury. This mutant is involved with the coding of the GDP-D-Mannose pyrophosphorylase enzyme[6] in the Smirnoff-Wheeler pathway for ascorbate biosynthesis. Smirnoff et al. (2001) also suggested that other pathways can produce ascorbate without relying upon the pyrophosphorylase step, but most probably at a slower through-put rate, because any fully ascorbate-deficient mutant would be lethal, perhaps because of ascorbate use as a cofactor rather than its antioxidant properties. In addition to its lowered antioxidant capacity, the ascorbate-deficient *Arabidopsis* mutant vtc1 (Conklin et al., 1996; Conklin and Barth, 2004) may show suppressed growth due to lower mannose levels that are necessary for cell wall formation. Ozone thus may suppress growth in these mutants through interference with cell wall biosynthesis as well as through lower antioxidant protection.

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

[6] EC 2.7.713, Mannose-1 phosphate guanylyltransferase; mannose + GTP → GDP-mannose + ppi; this product leads into cell wall polysaccharide synthesis and protein glycosylation through GDP-galactose and GDP-fucose and, ultimately, through galactose into ascorbate synthesis.

transporter. Pathway II uses a direct transporter of ascorbate/DHA. Pathway III moves the required electron(s) through a cytochrome b system, maintaining two separate pools of ascorbate/DHA (within the cytoplasm and within the wall). Each of the pathways (Dietz, 1997) represented by Roman Numerals in the Figure AX9-12, require only one NAD(P)H molecule to reduce the DHA molecule back to ascorbate. However, the transport properties and redox potential of the cell differ for each pathway. The efficiency of the reduction of DHA is dependent upon the redox coupling and the region in which the chemical species is located.

Turcsányi et al. (2000) exposed broad bean (*Vicia faba*) grown under two regimes in duplicate controlled chambers: charcoal/Purafil filtered air (CFA) or (CFA) plus 0.075 ppm O_3 for 7 h/day for 28 days (chronic exposure) or exposed to 0.150 ppm for 8 h (acute exposure). Responses of the two sets of plants were similar except for stomatal conductance, which was 50% lower in the chronically exposed plants. Plants grown under acute exposures developed visible injury, while plants grown under chronic conditions developed no visible injury. Within an hour of the start of the acute exposure, the stomatal conductance was reduced by nearly 40% and assimilation was reduced by nearly 18% in the clean air plants; the reduction in conductance was only 21% and assimilation 16% in the plants subjected to chronic O_3 exposures. The assimilation was affected similarly in both cases, while the conductance showed less of a percentage drop in the chronic O_3-exposed plants and began at a lower O_3 level. The similarity of the assimilation indicated that the stomata were not limiting assimilation in either case before acute exposure. More to the point, the decline in ascorbate in the apoplastic space due to the O_3 exposure was "…more often than not, on the borderlines of statistical significance." However, a 30% decline in ascorbate after 4 h of acute O_3 exposure in both cases was observed. This lack of significance may be due to a relatively large standard error of the data, which in turn may be due to the difficulty of extracting and measuring ascorbate from the apoplastic space in quantitative terms.

The chemical reaction[7] of ascorbate and O_3 is given by the molecular rate constant of $4.8 \times 10^7\ M^{-1}\ s^{-1}$. This is some 50,000× the rate constant for ET. Of course, it depends upon the relative concentration of ascorbate and ET, but it is likely that ascorbate is in higher

[7]These chemical rate constants are those constants within a bulk solution. In the apoplasm, the possibility exists for the chemicals to be preferentially oriented near a surface; so the constants may not be the same as for bulk solutions.

concentration than ET. One would then expect that the rate reaction of ascorbate with O_3 would greatly dominate any possible reaction of O_3 with ET. For a concentration of ascorbate in the range of 1 mM and for an O_3 concentration of about 0.1 ppm or 4.2×10^{-9} M, the detoxification rate would be $4.8 \times 10^{7} \times 10^{-3} \times 4.2 \times 10^{-9}$ M $s^{-1} = 2.0 \times 10^{-4}$ M s^{-1}. Turcsányi et al. (2000) calculated an O_3 flux of about 1.6×10^{-9} moles m^{-2} sec^{-1}. With a wall thickness of 0.12×10^{-6} m and all the O_3 flux going into the wall region, this would give about 1.3×10^{-2} mol m^{-3} s^{-1} or 1.3×10^{-5} M s^{-1} flux, which is less than 10% the detoxification rate.

Glutathione

Many of the initial studies of O_3 exposure used high concentrations and measured only the total sulfhydryl contents of the tissues. For example, in some of the earlier work, exposures of tobacco to 1 ppm O_3 for 30 min induced a 15% loss of the total sulfhydryls (0.74 μmole/g-FW) (Tomlinson and Rich, 1968). These results are similar to other studies at high O_3 levels (Dugger and Ting, 1970). It is now suspected that the severe injury in their studies resulted in a massive collapse of the cells and release of most of their internal constituents. Much of the oxidation thus observed may have been the result of chemical oxidations of the O_3 that subsequently entered the damaged tissue. Even under milder conditions, changes in sulfhydryl components have still been noted and any sulfhydryl on the surface of the cell would be at risk due to its high reactivity with O_3 (Mudd et al., 1997b; Mudd et al., 1969). For example, the level of sulfhydryl compounds within the protein of isolated chloroplasts declined about 66% when the chloroplasts were subjected to O_3 (about 1 μmole O_3) exposure (Mudd et al., 1971).

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

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

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

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

Catalase

Catalase, even though it breaks down H_2O_2, does not appear to protect plants from O_3 exposures. Two principal reasons may cause this lack of reactivity: (1) catalase has a high Km for H_2O_2 and a low rate coefficient, and (2) catalase seems not to occur within the cell wall regions but rather in the cytoplasm and peroxisomes (Buchanan et al., 2000). While a few reports suggest that catalase is increased by exposure to O_3 (Azevedo et al., 1998), Booker et al. (1997) found no effect of catalase activity in soybean until late in the growing season, and others have found decreased catalase activity in wheat in response to O_3 (McKee et al., 1997). Unfortunately H_2O_2 induced by some forms of wounding in mesophyll cells can lead to induction of an increase in GSH and the transient production of catalase (Vanacker et al., 2000). In general, it seems that catalase is not really involved primarily in the defense of the cell due to O_3 attack but rather may be a secondary response. The reaction of catalase (Scandalios, 1993) is as follows:

---

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

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

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

Superoxide Dismutase

The varied compounds that O_3 can produce upon entering an aqueous solution are very similar to those involved in the HR when plants are infected by an avirulent pathogen (Figure AX9-10). The sequence of the plant response to the pathogen is (1) recognition of the gene products of the pathogen by the plant (elicitor), (2) generation of an immediate phytoresponse to attempt to localize the attack and its products, and (3) generation of a systemic acquired resistance (SAR) to subsequent attack by the pathogen. Inducible defense responses are phytoalexin synthesis and production of pathogenesis-related proteins (PR). One aspect of this total response is the production of O_2^- and H_2O_2 by the cell (Lamb and Dixon, 1997). The elicitor can generate a transient alkalinization of the apoplast, up to pH 7.2, caused by a lowering of the H^+-pump rate and an increase in the H^+-influx/K^+-efflux exchange. Other effects include a weak accumulation of transcripts for PAL (phenylalanine lyase); a larger and rapid induction of glutathione S-transferase, GSH-P_x; oxidative cross linking of cell wall proteins which is blocked by ascorbate acid; generation of localized apoptosis; and rapid influx of Ca^{2+}, which activates apoptosis among other pathways (Lamb and Dixon, 1997). These effects seem to be very similar to those induced by O_3 exposure (Sandermann, 1996, 1998).

The putative antioxidant enzyme SOD (Equation AX9-4 and Table AX9-8) catalyzes the oxidoreductase reaction, which eliminates SO_2^- by dismutation (Bowler et al., 1992):

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

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

Table AX9-8. Superoxide Dismutase Isozymes and Isoforms

Reaction: $2H^+ + 2\ O_2^- \rightarrow H_2O_2 + O_2$			
Isozymes	**M.W.**	**Isoforms**	**Cytolocation**
Cu-Zn	20 kDa	*csd1*	
		csd2	Plastid
		csd3	Peroxisomal
Fe	23 kDa	*fsd1*	Mitochondrial
		sd2	
		fsd3	Plastid
Mn	23 kDa	*msd1*	Mitochondrial

exposure of plants to 8 h of 0.33 ppm of O_3. *csd1* induced by O_3 and UV-B was one of the earliest SOD increases and most pronounced responses for mRNA and protein. Also, some increase in *csd3* (thought to be peroxisomal) was induced when the plants were exposed to a high-intensity light pulse; *msd1* was unresponsive to the environmental stressors used here, including O_3; and *csd2* (thought to be chloroplast) showed little increase. The *fsd1* isozyme (present in the apoplasm) showed a slight decrease. On the other hand, an early report on snap beans in which the experimenters used EDU, N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N-phenylurea (Carnahan et al., 1978; Kostka-Rick and Manning, 1993), to prevent visible injury by O_3, 4-h O_3 exposure at 0.45 ppm was correlated with an increase in general enzyme activity of SOD, i.e., the level rose nearly 2.5× in 2 weeks at a level of 50 mg EDU per pot (Lee and Bennett, 1982). It is believed that EDU may induce SOD, which then protects the plant. While gross assays of enzyme activity have not proven to be very useful in understanding the mechanism of O_3 action, in a well-crafted, long-term study involving ponderosa pine clones. Benes et al. (1995) stated that "changes in antioxidant enzyme activity were not a consistent response to the O_3 fumigation, but when observed, they occurred most often in the O_3-sensitive clone and in symptomatic, fumigated branches…total (intra- and extracellular) activities of the antioxidant enzymes did not appear to be good indicators of O_3 tolerance…."

Ozone exposure (70 ppb for 7 h/day for 14 to 42 days of exposure)[10] caused an increase in POD and a decline in SOD with no change in APX. No GSH was detected, but the concentration of (ASC + DHA) was at 20 to 25 nmol/g-FW of extracellular fluid, compared with 2.4 to 3.0 mmol/g-FW of cell fluid. Glutathione within the cell was only 100 to 170 nmol/g-FW of cell fluid. While these results are what one might expect for POD, the decline in SOD and lack of change in APX are not what would be expected if protection was provided by SOD and ascorbate. Yet as noted, because the rate of SOD reaction is many times higher than the rate of O_3 entry, there may be no pressure to increase the SOD level.

Some protection against visible injury (induced by 59 ppb daily mean O_3 for 14 h/day for 7 days) was observed in genetically modified tobacco plants with excess chloroplast SOD[11] (2 to 4 times higher), but less protection was observed in plants that had an excess of mitochondrial SOD (8× higher) (Van Camp et al., 1994). In all lines, the conductance of the leaves dropped about 50%, compared with the unmodified plants. There was a correlation with age of leaf (less injury in younger leaves) that corresponded to that found in spruce trees in which the amount of SOD declined in relation to the longer that needles were held on the tree (Polle et al., 1989). A slightly different study, however, found no O_3 protection with varied SOD within the needles (Polle and Rennenberg, 1991). Interestingly, in maize, the synthesis of SOD (any form) was not stimulated by O_3 exposure (at 0.50 or 0.75 ppm for 8 h, variable times thereafter) but was by exposure to 90% O_2 (Matters and Scandalios, 1987). It may be that this high level of O_3 does not affect the SOD, or perhaps it stimulates and degrades the enzyme simultaneously.

The conclusions to be drawn from these results are not obvious. There seems to be SOD (a Cu-Zn form) present in the apoplastic space of some plants, but it does not necessarily rise with O_3 exposure. Thus, either its concentration is sufficient to provide protection or it is not needed. Over expression of any SOD in other organelles may play a role, especially in the chloroplast (Cu-Zn or Fe forms); however, it may be playing a secondary role due to other effects of O_3 that generate conditions in which light can overload the chloroplast and generate

---

[10]At a level of 70 ppb, the concentration of O_3 in air was about 3.06×10^{-6} mol/m^3, which with the conductance of 0.042 mol/m^2 s, gives a flux rate of O_3 of 1.27×10^{-8} mole/^{m2} s. Converting the SOD rate of 23 units/g-FW into a SOD rate within the apoplastic space of 6.9×10^{-3} mol/m^2 s, or about 500,000 times the entry rate of O_3.

[11]The 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.

detrimental circumstances, including the production of SO_2^-. In addition, SOD is developmentally expressed in varied concentrations, so that long-term exposure to O_3 may alter each leaf's developmental age and, in turn, alter what level of SOD is observed. In any case, SOD does not seem to be the primary antioxidant system to protect against O_3.

Changes to the Plasmalemma

Reports of "peroxidation" generally occur within unicellular organisms subjected to very high levels of O_3 (in *Chlorella* [Frederick and Heath, 1970] and in *Euglena* [Chevrier et al., 1990]). Heath (1987) determined that by the time biochemical events were altered and MDA was produced in *Chlorella*, little permeability remained in the cells and most metabolic pathways were greatly disrupted by the subsequent loss of substrates. In fact, MDA production was concurrent with a high O_3 uptake by the cell, indicating a complete opening of the cell and associated with the concurrent inability to plate the cells on a glucose medium (indicative of cell death). Heath (1987) reached the conclusion that no one had proven that lipid oxidation was in any way a part of the initial reactions of O_3 with the cell, a conclusion confirmed by Mudd et al. (1997a). An excellent review regarding the initial action induced by O_3 within a plant (Kangasjärvi et al., 1994) should be consulted. There is little data to show that lipids are attacked by O_3 in any living system that was not previously severely injured by O_3. Most of the data suggesting lipid attack by O_3 has been demonstrated in plants subjected to O_3 concentrations of 0.5 to 1.0 ppm for several hours, during which gross wilting of the plant tissues usually occurs, suggesting extreme water loss. It is not surprising that lipid and protein injury are observed under these conditions. While those reports were useful in the 1960s and 1970s, they are not especially insightful now when ambient levels of O_3 are rarely above 0.2 ppm.

AX9.2.4 Wounding and Pathogen Attack

The decline of an enzyme is more difficult to measure than the rise of a new enzyme; an increase from 0 to 2% may be within the precision of any assay, but a decrease from 100 to 98% is often masked by simple variation of the assay. Thus, measuring enzymes, which are in great abundance in prefumigated tissue, is a risky operation. On the other hand, if O_3 induces a general physiological change that has characteristics similar to other well-studied, stress-induced

changes, then O_3 studies could "piggyback" onto those studies to gain insight into the full scope of metabolic alterations. It is now becoming clear that wounding and pathogen attack of plants are similar to O_3-induced changes in plants, and a reasonable hypothesis is that O_3 must induce one or more of the first steps seen in the wounding/pathogen-attack response.

Systemic acquired resistance (SAR) has been heavily investigated, and DNA probes have existed for some time for a series of expressed genes (see Table AX9-9). Several enzyme classes are associated with O_3 injury, including glucanases and peroxidases and others, such as the PR proteins and chitinases. Thus, strong evidence exists from enzyme function and genetic material that O_3 induces an activation of a SAR-like response.

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

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)

Mehdy (1994) described a model of how an elicitor produced by the pathogen attack activates a G-protein, which opens the inward-flowing Ca^{2+} channel. The flow of Ca^{2+} into the cytoplasm raises the internal level (at the μM level) and activates a protein kinase that increases the activity of the plasma membrane NAD(P)H oxidase and generates O_2^-. Superoxide dismutase converts O_2^- into H_2O_2. Both O_2^- and H_2O_2 are responsible for the active oxygen species response, which is believed to be a defense mechanism to kill the pathogen. In this normal defensive reaction, a subsequent system induces either localized lipid peroxidation per se or a membrane lipase to produce jasmonic acid or inositol triphosphate, which act as secondary messages to activate the defense gene products.

Booker et al. (2004) found that G-proteins might be involved in the perception of O_3 in the extracellular region using *A. thaliana* G-protein null mutants. The activation of a passive inward flow of Ca^{2+}, e.g., by an O_3-induced response, would serve the same function as activation of the G-protein. Once the level of cytoplasmic Ca^{2+} rises, all else follows. It is suspected that exposure of plants to O_3 does just that, as Castillo and Heath (1990) demonstrated — the in vivo fumigation of bean plants both inhibits the outward-directed ATP-requiring Ca^{2+} pump and increases the passive permeability of Ca^{2+}. It was thought that the calcium transporter system has a sensitive sulfhydryl group which, if oxidized, would alter normal Ca^{2+} movements. In addition, Dominy and Heath (1985) observed that the K^+-activated ATPase (believed to be involved in K^+ transport) was inactivated by in vivo exposure to O_3 and that inactivation was traced to a sensitivity sulfhydryl. Mudd et al. (1996) argued that several amino acids are very sensitive to O_3, including any with an exposed sulfhydryl. Thus, the O_3-induced change in Ca^{2+} permeability may be the trigger to most, if not all, the wounding responses. However, the difficult problem of proving that the cytoplasmic Ca^{2+} change is the first event in O_3 injury remains.

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

AX9.2.4.1 Peroxidases

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

At the same time, others examined peroxidase reactions in general and found two types of peroxidases (designated as acidic or anionic and basic or cationic, EC 1.11.1.7, but also listed as EC 1.14.18.1). Many types of peroxidases are located in diverse organelles, and each seems to be activated by different conditions (e.g., pH for anionic and cation types and substrates such as guaiacol, syringaldazine, and ascorbate). Peroxidases belong to at least two groups, which catalyze two separate reactions: (1) the reaction of H_2O_2 with ascorbate to form DHA, discussed earlier (Thom and Maretzki, 1985), which is regenerated by plasma membrane electron transport using a dehydrogenase (Gross and Janse, 1977) now believed to be a malate/oxaloacetate shuttle through the membrane coupled to a NAD(P)H-cytochrome-b-reductoxidase; and (2) the reaction with coniferyl alcohol (from phenylalanine through phenylalanine ammonia lyase) to form lignin within the wall. The anionic peroxidase thought to be involved with lignification is within the cell wall (Buchanan et al., 2000; Taiz and Zeiger, 2002). Some basic peroxidases are maintained within the cell, while some are external to the cell. After wounding (Gasper et al., 1985; Lagrimini and Rothstein, 1987), some basic peroxidases can be activated by processes leading to the synthesis of stress ET (Yang and Hoffman, 1984) and/or by excess Ca^{2+} (Gasper et al., 1985). Elicitor treatment of plants change a series of peroxidases, some of which are similar to those seen in O_3-induced changes (see Table AX9-9).

The formation of lignin is due to the phenylpropanoid metabolism (Buchanan et al., 2000). Tyrosine and phenylalanine are converted to cinnamic and *p*-coumaric acid, which are, in turn, converted to *p*-coumaryl, coniferyl, and sinapyl alcohols, and then into lignins. Hence, the peroxidase activity is often measured by one of these substrates (Espelie et al., 1986; Gasper et al., 1985). However, it is questionable whether apoplastic peroxidase activity is limiting for lignification; laccases have a prominent role as well. Also, availability of monolignols is critical for core lignin formation, and it is unclear whether levels of these metabolites change in response to O_3. Studies by Booker and others (Booker et al., 1991, 1996; Booker, 2000; Booker and Miller, 1998) indicated that O_3 did not increase core lignin concentrations in foliage of loblolly pine, soybean, or cotton; although levels of phenolic polymers and cell wall-bound phenolics were elevated in soybean. Increased phenolic polymers appear to be lignin in acid-insoluble lignin assays and may well be responsible, along with polyphenol oxidase, for the stippling injury observed in O_3-treated plants. Cell wall function implies the transport of peroxidase molecules out of the cell and, most likely, the regulation of their activities within the wall space. These extracellular peroxidases may be observed by vacuum infiltration of buffer into leaf air spaces and subsequent centrifugation of the tissues to remove the buffer with the apoplastic enzymes that wash out (Castillo and Greppin, 1986). However, exposure to O_3 induces important changes in the plant. For example, extracellular peroxidase activity in *Sedum album* leaves increased nearly 3-fold over that in the control plants after 2-h exposure to 0.40 ppm O_3 (Castillo et al., 1984). This O_3-induced increase in extracellular peroxidase appears to be under the control of Ca^{2+} (Castillo et al., 1984; Heath and Castillo, 1987). Initially, no effect on the anionic activity as measured with syringaldazine (specific electron donor for lignifying peroxidases) was observed, yet 21 h later, the anionic peroxidase activity was increased, whereas the cationic (ascorbate measured) peroxidase activity was decreased, in O_3-treated plants. This suggests an immediate response (ascorbate peroxidase activation) and a secondary response that activates the lignifying peroxidase via gene activation.

The rapid response of cationic peroxidase after O_3 exposure may not result from de novo protein synthesis but from the secretion and direct activation by Ca^{2+} ions of enzyme molecules already present in the tissue. Cationic peroxidases might attack the peroxides and, in this manner, act as a detoxifying agent with ascorbate as the substrate in the apoplasm. The effect of Ca^{2+} upon peroxidase activity is stronger at low H_2O_2 concentrations (Penel, 1986). Thus, one

can imagine that, when the H_2O_2 concentration is low, this peroxidase activation would have a greater in vivo importance. Furthermore, the secretion of cationic peroxidases into the free spaces as a result of O_3 treatment is accompanied by a simultaneous release of at least one of its natural substrates (ascorbic acid); this cationic peroxidase exhibits a much higher affinity toward ascorbate (up to 6-fold) than the anionic isozyme (Castillo and Greppin, 1986).

AX9.2.4.2 Jasmonic Acid and Salicylic Acid

Jasmonic acid (JA) and salicylic acid (SA) are considered to be regulators of the plant defense response (Figure AX9-13) (Buchanan et al., 2000). They tend to respond more slowly than ET, causing widespread effects in the plant tissues. Both seem to be heavily involved in responses of the plant to O_3, once again linking the pathogen/wounding defense to O_3-induced injury; however, their roles are far from clear.

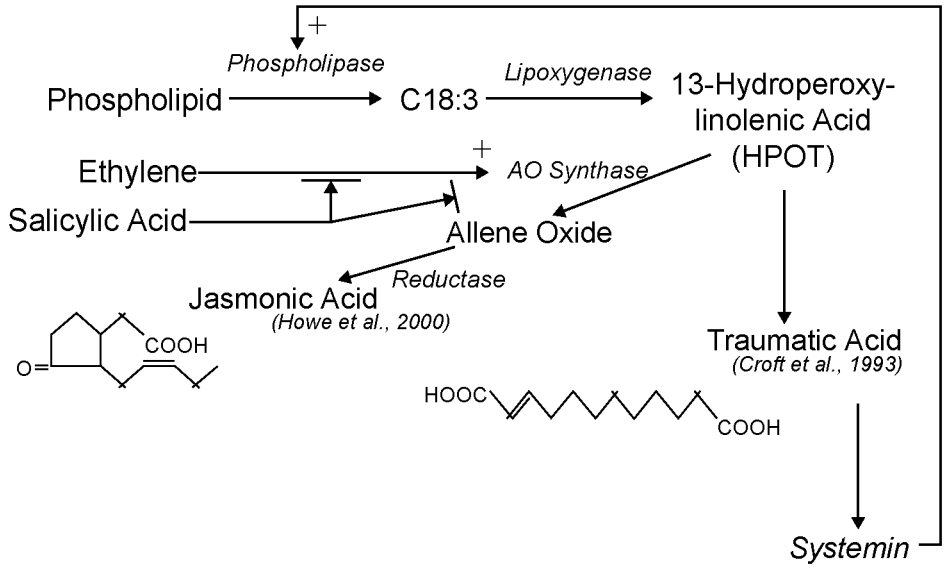


Figure AX9-13. The pathway leading from phospholipids to jasmonic and traumatic acid. The role of lipoxygenase and the production of a hydroperoxyl moiety from the unsaturated fatty acid are clearly demonstrated. More importantly, several of the enzymes within this pathway have been shown to be activated by oxidative conditions, including O_3 exposure. The production of both of these acid species could lead to a general global response of a whole plant to the O_3 exposure of a single leaf.

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

One of the lipoxidase isoforms is activated by pathogen infection (POTLX-3) within 6 h and accumulates for a week (Kolomiets et al., 2000). This enzyme is the first stage of the JA pathway which leads to 13-hydroperoxide linolcenic acid (HPOT) which is converted either to allene oxide through AOS or to C6 aldehydes through hydroperoxide lyase. These aldehydes act as signaling agents via systemic (Sivasankar et al., 2000) or volatile odiferous compounds (oxylipins) that have been implicated as antimicrobial toxins (Froehlich et al., 2001). Interestingly, these compounds seem to target the chloroplast envelop where they interact with its metabolism. As HPOT and AOs are both implicated in plant defense and are activated by O_3, these interactions may be related to how chloroplast enzymes and their mRNAs are involved in O_3-induced injury.

AX9.2.4.3 Stress-Induced Alterations in Gene Expression

Early studies addressed the qualitative and quantitative effects of O_3 on protein metabolism (Harris and Bailey-Serres, 1994). Subsequent reports suggested that the physiologic and metabolic consequences of exposure to O_3 were, in part, mediated by increased gene expression. A summary of the gene-linked changes in proteins induced by SAR was provided earlier in Table AX9-9. Of particular note are the productions of PR proteins, chitinase, glucanase, and acidic peroxidases that appear to be common markers used in many O_3 studies. A summary of varied proteins as measured by changes in the mRNA in *A. thalania* induced by O_3 exposure is shown in Table AX9-10. While studies on *Arabidopsis thaliana* required high concentrations of O_3 to produce a response, the levels reported in most of the studies did not induce visible injury. The types of messages induced included glutathione S-transferase, PAL, ACC synthase, SOD, and some PRs. Slower increases in messages are seen for other PR and SAR-senescence proteins. Declines in messages were observed for varied chloroplast enzymes, including those for Rubisco and chlorophyll binding proteins. A few new proteins were found — a casein kinase and three plasma membrane proteins. It is interesting to note that few messages for "new" proteins were generated by O_3 exposure.

The working hypothesis is that O_3, which is not eliminated by antioxidants in the cell wall, alters the properties of the plasma membrane. Specific polypeptides, indicative of these antioxidants, are induced. If specific receptor molecules or channels on the membrane are affected, the ionic balance within the cytoplasm is changed, leading to altered transcription or

Table 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, *rbcS*	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-SODl 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					rcd1, on chromosome 1, single Mendelian trait	Overmyer et al. (2000)

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

GST = Glutathionine synthase
PAL = Phenylalanine ligase
PR-1 = Promoter region 1
Pxase = Peroxidase
CAT = Catalase
LOX1 = Lipoxygenase
ACS-6 = ACC synthatase
SOD = Superoxide dismutase
CuZn-SOD = cyto SOD
Fe-SODl = Chl SOD
cyt APX = Ascorbate peroxidase
Chl GPX = Glutathione peroxidase
Cab mRNA = Chlorophyll a/b binding protein
chl GR = Gluthatione reductase
BCB = Blue copper binding protein
ERD1 = Ethylene response
SAG21 = Senescence
rbcS = Rubisco small subunit
MT1 = Mitochondria

translation of the genes controlling those and other types of polypeptides. Once this membrane disruption occurs, the cell must mobilize repair systems to overcome the injury. Thus, carbon and energy sources once destined for productivity, must be used in repair processes. Some of these repairs are thought to result from the induction of specific genes. Photosynthesis is inhibited by direct inhibition of some of the enzymes, through byproducts of O_3 attack or by altered ionic balance. At the very least, the decrease in photosynthesis is a result of an O_3-induced decrease in *rbcS* mRNA.

AX9.2.5 Primary Assimilation by Photosynthesis

AX9.2.5.1 Photooxidation: Light Reactions

Photooxidation refers to the oxidation of chlorophyll within the light reaction due to an imbalance between light absorption and the CO_2 use to produce carbohydrates. It was discovered in the 1920s and studied under the concept of chlorophyll bleaching and photo-autooxidation (Asada, 1999; Rabinowitch, 1945). What generally occurs is that electron transfer from H_2O to NADPH declines, and a light reaction overload occurs. The slowdown of electron transfer may also be due to inhibition of the dark reactions, through the poor use of small molecular weight carbohydrates or a lowered amount of the fundamental substrate CO_2. To counteract these detrimental reactions, a series of "antioxidant" reactions exist, which eliminate the buildup of oxidative intermediates.

A lowered CO_2 level, which can be caused by stomatal closure (Heath, 1996), blocks the use of reduced plastoquinone (PQH_2) in Photosystem II through NADP reduction in Photosystem I (Hankamer et al., 1997). The buildup of PQH_2 reduces the amount of Q_A, resulting in a buildup of $P_{680}^+|Pheo^-$ species (the primary photoact). The inability to reduce this radical leads to injury to the D_1 protein (32 kDa) and its fragmentation into 23-, 16-, and 10-kDa fragments (Hankamer et al., 1997). Ozone exposure of bean plants leads directly to the loss of this D_1 protein (Pino et al., 1995). The loss of D_1 stimulates the production of new D_1 (and its mRNA). Also, the production of the oxidized form of P_{680} (P_{680}^+) is harmful to the plant, because electron flow from water to P_{680}^+ is limited, generating a P_{680}^T (the triplet form of P_{680}), which is highly oxidizing and can lead to dangerous reactions. One form of protection is the use of β-carotene to convert the triplet form back to its normal state; however, that reaction can lead to the loss of β-carotene. Without the protection of β-carotene, oxygen reacts with oxidized

products to produce singlet state of O_2. This, in turn, can react with chlorophyll, leading to ring breakage that, in essence, leads to chlorosis. These types of reactions do not seem to occur often, but chlorosis is one form of visible injury, and loss of β-carotene has been reported.

Using a FACE system to expose soybean to elevated O_3, Morgan et al. (2004)found that, in leaves at the top of the canopy, there was no effect on the maximum light-adapted apparent quantum efficiency of PSII (ϕPSII,max), electron transport at growth [CO_2], and saturating light (J_{sat}) nor on the probability of an absorbed photon reaching an open PSII reaction center (F_v'/F_m'; the quantum yield). There was a small, but significant, decrease in photochemcial quenching (qP) at the top of the canopy. As leaves aged, the decrease in qP was significant as leaves began to senescence, likely due to losses of chlorophyll. The results of these studies and others suggest that alterations to the dark reactions are much more common than to light reactions (Farage et al., 1991; Farage and Long, 1999).

AX9.2.6 Alteration of Rubisco by Ozone: Dark Reactions

A large body of literature published since 1996 shows that O_3 exposure affects Rubisco concentrations (Pell et al., 1997). Treatment of a variety of plants with O_3 at near-ambient levels results in a loss of Rubisco and of the mRNA coding for both subunits of Rubisco (*rbcS*, small and *rbcL*, large). In addition, oxidation of Rubisco by O_3-generated ROS may be an important factor in suppressing photosynthesis (Pell et al., 1997). Increased carbonyl concentrations of Rubisco are correlated with O_3 injury (Kanoun et al., 2002; Leitao et al., 2003). Because Rubisco plays such an important role in the production of carbohydrates (Figure AX9-14), any loss may have severe consequences for the plant's productivity.

Chronic O_3 exposure, both with and without elevated CO_2, significantly lowered assimilation and leaf conductance of soybean in aging mature leaves (Fiscus et al., 1997; Reid and Fiscus, 1998), which was associated with significant decreases in Rubisco content in aging leaves. Noormets et al. (2001b) also found that O_3 had the greatest effect on older leaves of aspen clones using a FACE exposure facility in which areas of ambient CO_2 (daytime 360 ppm) and ambient with added CO_2 (560 ppm), with added O_3 (97.8 ppb), and with added CO_2 and O_3, were used. They found an O_3- induced decline in assimilation and in conductance, and subsequently confirmed that the internal CO_2 (calculated for within the leaf) is not affected by O_3 exposure. Higher levels of CO_2 increased the assimilation and lowered the conductance,

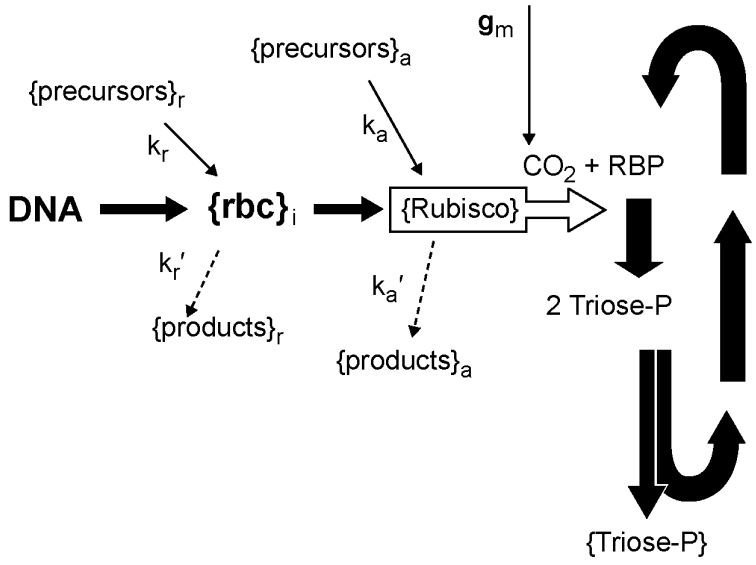


Figure AX9-14. The production of Rubisco and its Calvin Cycle pathway reactions. Two peptides are used to build Rubisco: *rbcS*, the small subunit produced by DNA within the nucleus; and *rbcL*, the large subunit produced by DNA within the chloroplast itself. Clearly both polypeptides must be closely regulated to produce the enzyme in a coherent manner. Furthermore, at least five isoforms of DNA can produce *rbcS*, each of which is regulated by a different promotor region.

maintaining the internal to external CO_2 ratio identical to that found with the ambient CO_2 level, corresponding to the theory of Farquhar et al. (1980). More to the point was that the stomatal limitation[12] was not altered by O_3 exposure, with or without excess CO_2. It is critical to point out that mesophyll conductance is directly linked to internal CO_2 level[13]. So if C_i/C_o ($C_o = CO_2$ outside) is constant and g_s declines, then g_m must likewise decline. If, as it is argued, Rubisco levels are constant or at least increasing, then a regeneration of RuBP must be the cause of the

[12]The limitation was defined as the ratio of stomatal resistance to the total resistance, which included the operating point of the assimilation (A) verses internal CO_2 concentration (C_i) curve and the resistance of the boundary layer. The operating point of the curve was defined as the internal CO_2 level, which is calculated by the conductance and assimilation. The resistance of this operating point was calculated as the cotangent of the slope to the operating point. Unfortunately, the slope is not a dimensionless parameter but is rather moles of air per area of leaf $^{-}$s of time and, thus, it is unclear whether the slope changes with added CO_2 and O_3.

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

decline in g_m. Farquhar et al. (1980) were more concerned with high levels of CO_2 and had little to say about O_3 exposure.

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

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

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

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

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

Williams et al. (1994) developed a correlation between the levels of ABA after water stress in *Arabidosis thaliana* leaves and the loss of *rbcS*. Although their data were not quantitative, the level of ABA had a half-time rise of about 1 to 2 h and the level of *rbcS* had a half-life decline of about 2 to 4 h. Their work suggests that drought stress may alter the CO_2 metabolism by changing enzyme relationships much more than by merely closing the stomata. If an ABA rise is lowering *rbcS*, *rbcS* may not be a good marker of O_3 fumigation except under highly controlled conditions.

AX9.2.7 Carbohydrate Transformations and Translocation

The question of whether translocation of the sugars out of the leaf is inhibited by O_3 exposure arises, because productivity is often dramatically inhibited by O_3 fumigation. Though nearly 35 years have passed since Dugger and Ting (1970) investigated the question of sugar transport within the leaf, the question has since been little studied. Translocation (Cooley and Manning, 1987) appears to be inhibited, because root functions are impaired by O_3 exposure. Many observed events suggest that while carbon assimilation within the leaf declines, translocation of carbon is inhibited even more so, because plant growth points are inhibited and root/shoot ratios are altered (Dugger and Ting, 1970; Gerant et al., 1996; Tjoelker et al., 1995).

Many of the experiments with O_3 fumigation indicate that O_3 exposure decreases the net growth or dry mass of the plant, but the mechanism is poorly understood. Generally the decrease in assimilation is much less than the decrease in growth, but not always. Under many conditions, the stomata will close partially, decreasing assimilation by a smaller factor. Only a long exposure, or high levels of exposure for a short time, generate enough decline in Rubisco to make the growth of the plant problematical. No convincing argument has linked the decrease in growth with a small decline in assimilation, either by a conductance- or Rubisco-limitation. Measures of assimilation with crops are frequently done on upper canopy leaves, which are the last leaves to exhibit O_3 injury, while leaves deeper in the canopy exhibit injury and early senescence. Crop root growth must be sensitive to these and other O_3 effects, because root biomass is often suppressed early by elevated O_3.

Volin et al. (1998) found O_3 exposures statistically decreased leaf area ratio, specific leaf area, leaf weight ratio, and root weight ratio in *Populus tremuloides* and two C3 grasses (*Agropyron smithii* and *Koeleria cristata*) but not in *Quercus rubra* and in the C4 grasses *Bouteloua curtipendula* and *Schizachyrium scoparium*. There was no statistically significant change in any species in leaf conductance (4% level decline in *K. cristata*) nor in assimilation (although there was a decline in assimilation at the 6% level for *P. tremuloides* and a decline at the 1% level in *B. curtipendula*). They also reported a correlation between growth decline and decreased stomatal conductance among all species.

Tradeoffs are made by plants. Birch grown in highly fertilized conditions exhibited a greater leaf turnover when exposed to O_3, in that leaves not only formed faster but abscised faster, presumably due to early senescence; whereas birch grown under poorer fertilized conditions retained their leaves longer and had a greater respiration rate within those leaves (Maurer and Matyssek, 1997). Again, one must be careful in comparing short-term versus long-term exposures. Grulke et al. (2001) observed that maximum concentrations of carbohydrates in 1-year-old needles that had not abscissed due to early senescence declined when subjected to year-long exposures along an increasing pollution gradient. Furthermore, the monosaccharide concentrations (along with starch) in fine roots were largely decreased, suggesting that needle sugars were limiting, leading to root-sugar limitations. However, determination of the total productivity and detailed balance of carbohydrate was impossible, because these were older, larger trees and the data were taken over a full growing season. For a shorter-term exposure of 9 days, Smeulders et al. (1995) observed that O_3 appeared to increase the retention of labeled photosynthates within the needles of Douglas Fir, and, at higher exposures (400 versus 200 or 0 $\mu g/m^3$), the total starch within the needles decreased, suggesting that less carbohydrate was produced within the cell or perhaps that it was in compounds not measured.

Studies with Pima cotton (*Gossypium barbadense*), aspen (*Populus* spp.) and bean seedlings (*Phaseolus vulgaris*) indicate that acute O_3 exposures inhibit export of the current assimilate that provides carbohydrates to the roots from source leaves of cotton as well as recent assimilate from the older leaves of aspen and bean (Grantz and Yang, 2000). Grantz and Yang (2000) attempted to distinguish between potential mechanisms of O_3 phytotoxicity operating at the level of the whole plant. Four hypotheses were tested by fumigating cotton: (1) O_3 exposure reduces leaf pools of soluble sugars; (2) pruning leaf area and reducing source strength to match

that of O_3-treated plants reproduces O_3 effects; (3) pruning lower leaf area more closely reproduces O_3 effects than pruning the upper leaf area; and (4) manipulating plant age and, thereby, plant size to match O_3-treated plants reproduces O_3 effects. All were shown to be incorrect. Under each of the above conditions, Grantz and Yang (2000) reduced the amount of foliage to match that caused by O_3 injury. While the treatments reduced the biomass and leaf area, they did not alter biomass allocation nor root function. They concluded that a simple loss of foliage does not induce the changes in translocation to the roots to the same extent as does O_3 injury.

This finding by Grantz and Yang (2000) is important in that it suggests that O_3 can trigger a plant-wide response that may be linked to alterations in signal transduction and the generation of whole plant signals. Stitt (1996) suggested that "…allocation is regulated by long-distance signals that act to influence growth of selected sinks and to modify the delivery of resources to these sinks in parallel." Cooley and Manning (1987), citing McLaughlin and McConathy (1983), suggested three possible ways that O_3 fumigation might alter translocation: (1) malfunction of the phloem loading process, (2) increased translocation to leaf injury repair, and (3) an altered balance between the leaf and sinks caused by reduced carbon fixation and a greater demand for assimilate in the leaf.

Ethylene has been shown to reverse this sugar inhibition of development and to be antagonistic to the ABA effect (Finkelstein and Gibson, 2002). However, these effects depend greatly upon the developmental stage of the plant. Thus, the balance of the effectors (sugars, ABA, and ET) may interact to generate the variation observed in the O_3-induced productivity decline. For example, O_3 fumigation can induce a shift in the carbon transfer between roots and shoot, and this shift can be amplified by mild drought (Gerant et al., 1996). Furthermore a regulation of source-sink relations with the defense responses induced by elicitors was observed by wounding the leaves of *Chenopodium rubrum* (Roitsch, 1999). Ethylene appears to be able to repress the expression of extracellular invertase, which is critical for control and downloading of sucrose derived from the translocational stream (Roitsch, 1999). In addition, the development of *Arabidopsis* at high concentrations of glucose or sucrose is arrested by increasing the ABA level (Coruzzi and Zhou, 2001).

Clearly more work is needed on the interactions between assimilation, translocation, and source/sink relations with O_3 exposure. In these interactions, one must be aware of the developmental age of the plants and their phytohormonal status.

AX9.2.7.1 Lipid Synthesis

Heath (1984) summarized several early reports of O_3-exposure induced lipid alterations. Most concerned the production of MDA (malonyldialdehyde) as a measure of lipid oxidation as well as the loss of unsaturated fatty acids. However, a series of experiments by Sakaki and coworkers (Sakaki et al., 1983, 1985) concentrated on one type of fumigation system and one metabolic pathway. This literature provides the best, most complete story with regard to lipid metabolism and O_3 fumigation and suggests that O_3 injures cellular membrane systems via lipid destruction.

Sakaki and coworkers (Sakaki et al., 1983, 1985) used spinach, which is a sensitive plant but which has not been much evaluated with respect to O_3 fumigation. While the O_3 level was high (0.5 ppm), enough work has been done to be able to discern what is happening. The first paper showed that chlorophyll bleaching does not begin until the plants have been exposed to O_3 for more than 10 h, whereas some MDA production begins with as little as 6 h exposure (Sakaki et al., 1983). Consistent production of MDA, indicative of gross disruptions, occurred only after 8 h exposure (Sakaki et al., 1985), within the timescale when chlorophyll and carotenoid levels begin to decline. Concurrently, the total fatty acid (FA) level decreased from ~481 to 358 nmol/cm^2 as the MDA level increased from 0.6 to 2.4 nmol/cm^2, indicating FA peroxidation (Sakaki et al., 1985).

Sakaki et al. (1983) also studied the development of changes by cutting disks from exposed leaves and floating them on water solutions for varied time periods (up to 24 h). This permitted feeding experiments to be done easily, whereas the cutting gives rise to an additional wound response and eliminates metabolite movement to and from other portions of the plant. The floating experiments indicated that, after exposure, scavengers of singlet oxygen ($^1O_2$), such as D_2O, and of hydroxyl radicals, such as benzoate and formate, have no effect on development of the MDA response after 8 h of in vivo fumigation, while scavengers of (O_2^-), such as tiron and ascorbate, lowered the amount of MDA formed. By measuring metabolites immediately after cessation of fumigation, they were able to show that ascorbate loss began with the onset of

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

Nouchi and Toyama (1988) exposed Japanese morning glory (*Ipomea nil*) and kidney bean (*Phaseolus vulgaris*) to 0.15 ppm O_3 for 8 h. Under these conditions, little visible injury was found with up to 4 h of exposure, while injury increased by ~50% after 8 h of exposure. Morning glory produced more MDA than kidney bean, which produced the same as the zero-time control. Morning glory also demonstrated a slight (5%) drop in MDGD (monogalactosyldiacylcerol), with increases in PC (phosphatidylcholine), PG (phosohatidylglycerol), PI (phosphatidylinositol), and PE (phosphatidylethanolamine) after 4 h. Twenty-four hours later, the drop in MGDG (mongalactosyldiacyglyerol) was much larger and was thought to be related to an inhibition of UDP-galactose galactotransferase due to a rise in free fatty acids (FFAs) in the chloroplast. Note that the two distinct timescales involved in O_3 fumigation, immediately postfumigation and a day or so later, allows for comparison after the plant metabolism responds to the fumigation event.

The pathway for the formation of MGDG and DGDG (digalactosyldiacylglycerol) is located on the chloroplast envelope. Diacylglycerol (DG) arrives from either the endomembrane system or the stroma and the enzyme UDP galactose-1,2-diacylglycerol galactosyltransferase (UDGT) forms MGDG with galactose from UDP-galactose. Sakaki et al. (1990) suggested that the O_3-induced inhibition of UDGT was due to a release of FFAs from within the chloroplast. These FFAs are inhibitory to UDGT, but not to GGGT, which is stimulated by high concentrations of Mg^{2+} (Sakaki et al., 1990). The Sakaki et al. (1990) data indicate that the in vivo measured activities of both enzymes isolated after fumigation are not affected by O_3 fumigation. Both enzymes have sensitivity sulfhydryls, and both are located on the envelope. Ozone, if it reaches those sulfhydryls, should inhibit these enzymes; yet inhibition was not seen.

It has been thought for years that tocopherols functioned as antioxidants in biological systems (Tappel, 1972). Hausladen et al. (1990) examined the role of antioxidants in red spruce (*Picea rubens*) by following seasonal changes. They fit the level of tocopherol within the needles (/g-FW) to the time of the year and found little change (fit as level = $A + Bt + Ct^2$).

From this empirical fit, they found that the constant A was lower with higher levels of O_3. The seasonal variation coefficients, B and C, were also lower, suggesting year-long low tocopherol levels. Variation with the season is not particularly surprising, given that phytochrome action may be linked to tocopherol biosynthesis (Lichtenthaler, 1977). Hausladen et al. (1990) reported a significant ($p < 0.05$) trend in the difference between the high and low level of treatment; although there was no discussion of why it occurred or what it meant in relation to metabolism. Their major conclusion was that the antioxidant changes due to O_3 exposure may decrease cold hardiness.

Sterols, believed to act as membrane stabilizers, have been investigated by several groups with mixed results. Tomlinson and Rich (1971), who exposed common bean at 0.25 ppm for 3 h, and Grunwald and Endress (1985), who exposed soybean at 0.07 ppm for 6 h for 48 days, reported an increase in free sterols and a decline in esterified sterols. However, Trevathen et al. (1979) exposed tobacco at 0.3 ppm for 6 h and reported opposite results. None of these investigators believed that O_3 had attacked the sterols directly, instead, they believed that these changes involved metabolism and membrane stability. If O_3 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 O_3 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 O_3 take longer to occur and tend to become most obvious under long periods of low-O_3 concentrations. These have been linked to senescence or some other physiological response very closely linked to senescence. These two responses, separated by a time sequence, are shown diagrammatically in Figure AX9-15.

The understanding of how O_3 affects long-term growth and resistance to other biotic and abiotic insults in long-lived trees is unclear. Often, the conditions to which a tree is subjected to in one year will affect the response of that tree in the next year. This has been called “memory effect”, although the term “carry-over” is preferred. In other words, a condition in an earlier year sets the stage for a reaction in the next year; thereby giving a “cause-effect” scenario.

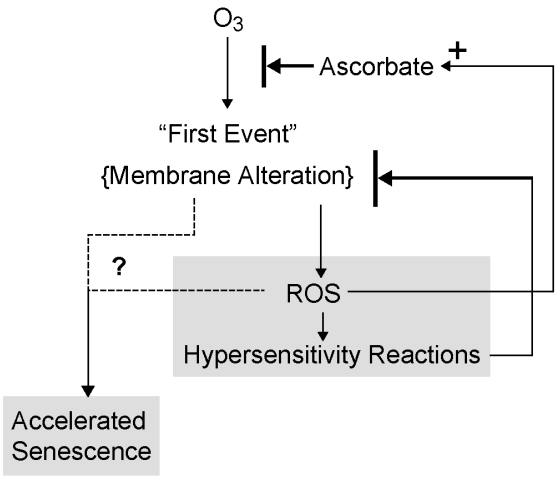


Figure AX9-15. Linkage of senescence with hypersensitivity reactions and the first event of O_3 attack of plants.

In perennial plant species, growth affected by a reduction in storage carbohydrates may result in the limitation of growth the following year (carry-over effects) (Andersen et al., 1997). Carry-over effects have been documented in the growth of tree seedlings (Hogsett et al., 1989; Sasek et al., 1991; Temple et al., 1993; U.S. Environmental Protection Agency, 1996) and in roots (Andersen et al., 1991; U.S. Environmental Protection Agency, 1996). Accumulation of the carry-over effects over time will affect survival and reproduction. Data on the cumulative effects of multiple years of O_3 exposures have been, for the most part, the result of 2- to 3-year seedling studies. The difficulty of experimentally exposing large trees to O_3 has lead to the tacit assumption that seedling response to O_3 is a good predictor of large-tree response to O_3 (U.S. Environmental Protection Agency, 1996).

The carry-over effects of O_3 exposures as observed in tree seedlings cited above by Hogsett et al. (1989) have been termed "memory effects" by Langebartels et al. (1997) and proposed by Schmieden and Wild (1995) to explain the sensitivity of spruce seedlings to frost in the winter after having been exposed to O_3 during the previous summer. Norway spruce (*Picea abies* L.) exposed to 80 ppb O_3 for a whole growing season, demonstrated visible injury symptoms the following year when the new needle flush appeared (Langebartels et al., 1997). Additional studies using Norway spruce and Scots pine (*Pinus sylvestris* L.) seedlings have shown similarly delayed responses following O_3 exposures. Carry-over symptoms were noted to develop at

different times of the year, depending on the species of seedling exposed: in early spring for Norway spruce, and in early autumn for Scots pine (Lange et al., 1989). Visible effects of O_3 exposures on spruce and pine may develop after a substantial delay during the "sensitive" periods of the year when chlorophyll and needle loss normally occur. Norway spruce and Scots pine differ in their sensitive periods because of the different needle classes normally remaining on the tree (Langebartels et al., 1997).

Nutrient status of the tree during the overwintering phase of its life (Schmieden and Wild, 1995) and chronic exposure to ambient O_3 (less severe with fewer peaks of very high levels) induce (1) mineral nutrient deficiency; (2) alterations of normal metabolism, including allocation of carbohydrates and probably nitrogen; and (3) disturbance of normal transpiration and diurnal cycling, leading to water stress. This condition, termed "Montane yellowing", appears to be related to nutrient deficiencies rather than senescence (although early loss of leaves and needles occurs). While generalized low nutrient concentrations may not occur within the foliage, localized deficiencies may. However, they are hard to observe or prove without a great deal of work involving all portions of a tree and without a general hypothesis of what is occurring.

AX9.2.9 Summary

As the understanding of wounding responses of plants and more genome details and varied plant mutants become available, the cellular and physiological responses of plants to O_3 exposures are slowly becoming clearer. However, more studies are needed on a larger variety of species. Nevertheless, several key findings and conclusions can be highlighted:

(1) The entrance of O_3 into the leaf through the stomata remains the critical step in O_3 sensitivity. Not only does O_3 modify the opening of the stomata, usually closing it partially, but O_3 also appears to alter the response of stomata to other stressful situations, including a lowering of water potential and ABA responses. The concentration of O_3 within the leaf is not the same as the external concentration due to reactions within the leaf, but it is not "zero".

(2) The initial reactions of O_3 within the leaf are still unclear, but the involvement of H_2O_2 is clearly indicated. The detection of possible products by EPR spectroscopy has progressed, but has not reached the point where any products can be identified. Nonetheless, reaction of O_3 (or its product) with ascorbate and possibly other antioxidants present in the apoplastic space of the mesophyll cells is clear and serves to lower the amount of O_3 or product available to alter the plasma membrane of the cells.

(3) The initial sites of membrane reactions seem to involve transport properties and, possibly, the external signal transducer molecules. The alteration and mechanism of the alteration of the varied carriers of K^+ and Ca^{2+} is far from clear, but it would seem that one of the primary triggers of O_3-induced cell responses is a change in internal Ca^{2+} levels.

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

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

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

AX9.3 MODIFICATION OF FUNCTIONAL AND GROWTH RESPONSES

AX9.3.1 Introduction

The responses of plants to any air pollutant may be significantly influenced by a wide range of biological, chemical, and physical factors. A plant's genetic makeup is an important inherent biological determinant of its response, but response can also be modified by other biological agents such as disease-causing organisms, insects and other pests, and by other higher

plant species with which it may be competing for resources. Chemical factors that may influence response to an air pollutant range from mineral nutrients obtained from the soil to other air pollutants and agricultural chemicals. Physical factors that may influence response include light, temperature and the availability of moisture, which are components of climate and climate change.

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

The impacts of these various factors on plant response to O_3 and other oxidants were extensively reviewed in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). It was noted in that document that, since any combination of these factors may come into play at some time in a plant's life history, "response will be dictated by the plant's present and past environmental milieu, which also includes the temporal pattern of exposure and the plant's stage of development." That document also stressed that both the impact of environmental factors on response to oxidants and the corollary effects of oxidants on responses to environmental factors have to be considered in determining the impact of oxidants on vegetation in the field. The variability observed in plant responses to defined exposures to O_3, particularly under field conditions, is a consequence of the influences of genetics and the range of environmental variables.

In view of the large number of factors to be considered, this section focuses mainly on situations in which there is clear evidence that environmental factors truly interact with oxidant effects, i.e., they magnify or diminish the impact of O_3 and are *not merely additive* to it. Conversely, it will cover situations where O_3 acts synergistically or antagonistically, but not additively, with effects induced by other factors. It will also emphasize those interactions as a

result of which overall plant growth, development, and yield are adversely affected, rather than the details of interactions at the mechanistic level, unless the latter are deemed to be essential to an understanding of larger-scale effects.

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

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

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

The few models thus far used to investigate O_3 stress have been adapted from existing process models of crop or tree growth that include limited numbers of physical or chemical variables such as temperature, soil water stress, or nutrient deficiency. Taylor et al. (1994) provided a listing of several simulation models developed for trees at the individual, stand, and regional levels; these and many other models have been critically reviewed by Kickert and Krupa (1991) and Kickert et al. (1999). However, regardless of whether such models are descriptive/empirical or process/mechanistic, their outputs will always be associated with varying degrees of uncertainty and require validation against observable responses wherever possible. Kickert et al. (1999) also noted that very few of the models that have been described provide *risk assessments* that address likelihood, in contrast to *consequence assessments* that address the magnitudes of effects. Thus, even though capable simulation models of plant response to O_3 involving complex mixes of many biological, physical, and chemical factors may be out of reach at the present time, the use of newer mathematical approaches such as artificial neural networks (ANNs) has enabled insightful analyses to be performed in several field studies involving numerous micrometeorological and other environmental variables (e.g., Balls et al., 1996; Mills et al., 2000).

Because the ensuing subsections deal with studies of O_3 interactions involving an extremely wide array of biological, physical, and chemical factors in the plant's environment, it is inevitable that many different exposure facilities and regimes have been used in these studies. To provide specific information regarding the O_3 exposure concentrations, profiles, hours and days of exposure (as well as the types of systems and facilities used for the exposures) would add a wealth of detail that would do little to assist our understanding of the roles of environmental factors in modifying the impact of O_3 on vegetation or to facilitate our ability to estimate the magnitudes of any such modifications. Thus, only experiments in which the exposure levels and regimes were *within the bounds of ambient experience in North America* are discussed in the ensuing subsections, regardless of the type of exposure profile used. The cutoffs used have been ~200 ppb for peak hourly concentrations or for short-term exposures, ~100 ppb for daytime means involving prolonged exposures for several hours, or a doubling of ambient levels in cases in which enriched exposure levels were a function of ambient levels. Actual details of the exposure regimes and conditions can, of course, be obtained from the original references but are only stated here when any distinction is required between the effects of

different exposure levels. Hence, it should be understood that ensuing statements such as ". . .it was found that O_3 caused. . ." should always be read as ". . .it was found that exposures to O_3 [within the range of those that have been measured in ambient air] caused."

AX9.3.2 Genetics

The response of individual plants to O_3 is affected by several factors, including the environment in which it is growing, competition with neighboring plants, ontogeny, and genetics. This section examines the role of genetics in plant response to O_3. In addition, major knowledge gaps in the understanding of genetic aspects of O_3 response are pointed out.

It is well known that species vary greatly in their responsiveness to O_3 (U.S. Environmental Protection Agency, 1996). This again has been recently demonstrated for grassland species (Bungener et al., 1999b; Bungener et al., 1999a; Franzaring et al., 2002; Nussbaum et al., 2000a; Pleijel and Danielsson, 1997; Warwick and Taylor, 1995), wild herbaceous plants (Bergmann et al., 1999; Danielsson et al., 1999), agricultural crops (Benton et al., 2000; Elagöz and Manning, 2002; Fumagalli et al., 2001; Heagle and Stefanski, 2000; Köllner and Krause, 2003; Nali et al., 2002; Nussbaum et al., 2000b; Ollerenshaw et al., 1999; Postiglione et al., 2000; Renaud et al., 1997), horticultural shrubs and trees (Findley et al., 1997; Hormaza et al., 1996), and forest trees (Bortier et al., 2000a; Guidi et al., 2001; Landolt et al., 2000; Matsumura, 2001; Momen et al., 2002; Nali et al., 2002; Oksanen and Rousi, 2001; Pääkkönen et al., 1997; Pell et al., 1999; Saitanis and Karandinos, 2001; Volin et al., 1998; Zhang et al., 2001). These studies have shown a wide range of responses to O_3, from growth stimulation by a few species such as *Festuca ovina* L. (Pleijel and Danielsson, 1997) and *Silene dioica* and *Chrysanthemum leucanthemum* (Bungener et al., 1999b; Bungener et al., 1999a) to significant growth reduction, depending on environmental conditions and exposure dose.

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

1997; Pell et al., 1999). Volin et al. (1998) suggested that the relative rate of stomatal conductance and the photosynthesis rate at a given conductance both contribute strongly to determining a species sensitivity to O_3. Likely, there is more than one mechanism determining sensitivity, even in a single species.

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

AX9.3.2.1 Genetic Basis of Ozone Sensitivity

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

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

and Zilinskas, 1996). Transgenic tobacco plants expressing antisense RNA for cytosolic ascorbate peroxidase, which reduces ascorbate peroxidase production, showed increased susceptibility to O_3 injury, suggesting a key role in O_3 tolerance for the antioxidant ascorbate peroxidase (Örvar and Ellis, 1997).

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

While changing the expression of single antioxidant genes has proven very useful in identifying possible mechanisms of O_3 sensitivity and tolerance (Kuzniak, 2002), it should be noted that increased O_3 tolerance has not been shown in some studies of transgenic plants with enhanced antioxidant production (Strohm et al., 1999; Strohm et al., 2002; Torsethaugen et al., 1997). Clearly, ethylene production plays a role in O_3 sensitivity, but the roles of various antioxidants in O_3 tolerance regulation are yet to be fully elucidated (Wellburn and Wellburn, 1996). It is unlikely that single genes are responsible for O_3 tolerance responses, except in rare exceptions (Engle and Gabelman, 1966). Regulation of stomatal opening and leaf structure (Elagöz and Manning, 2002; Fujita et al., 1992) are likely to play key roles in O_3 tolerance in plants. Newly developing opportunities to examine simultaneous regulation of larger numbers of genes are also likely to yield more clarification of the genes controlling O_3 tolerance (Desikan et al., 2001; Matsuyama et al., 2002).

Attempts to demonstrate conclusive changes in antioxidant and protective pigments for O_3 sensitive and tolerant mature trees growing in the field have largely been unsuccessful (Tausz et al., 1999a,b). However, evidence for antioxidant expression differences contributing to differences in O_3 sensitivity of 4-year-old *Populus tremuloides* trees has been found (Wustman et al., 2001).

AX9.3.3 Environmental Biological Factors

The biological factors within the plant's environment that may directly or indirectly influence its response to O_3 in a positive or negative manner encompass insects and other animal pests, diseases, weeds, and other competing plant species. Although such interactions are ecological in nature, those involving individual pests, plant pathogens, or weeds, or agricultural crop or forest tree species are considered in this section. More complex ecological interspecies interactions are dealt with in Section AX9.5.

The different types of biological factors are dealt with separately, as in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). Still, it is important to recognize certain general features of relationships of plants with the biological components in their environments:

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

Ozone and other photochemical oxidants may influence the severity of a disease or infestation by a pest or weed, either by direct effects on the causal species, or indirectly by affecting the host, or both. In addition, the interaction between O_3, a plant, and a pest, pathogen, or weed may influence the response of the target host species to O_3. A perceptive overview of the possible interactions of O_3-exposure with insect pests and fungal diseases has been provided by Jones et al. (1994), based on a model system involving two insects and two pathogens affecting cottonwood (*Populus deltoides*). Their study also included effects on the decomposition of leaf litter.

In contrast to detrimental biological interactions, there are mutually beneficial relationships or symbioses involving higher plants and bacteria or fungi. These include (1) the nitrogen-fixing species *Rhizobium* and *Frankia* that nodulate the roots of legumes and alder and (2) the mycorrhizae that infect the roots of many crop and tree species, all of which may be affected by exposure of the host plants to O_3.

In addition to the interactions involving animal pests, O_3 may also have indirect effects on higher herbivorous animals, e.g., livestock, due to O_3-induced changes in feed quality.

AX9.3.3.1 Oxidant-Plant-Insect Interactions

The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) stressed the variability in the reported effects of O_3 on host plant-insect interactions. Since relatively few plant-insect systems have been studied, few consistent patterns of response have emerged, as noted in other reviews such as those of Colls and Unsworth (1992), Heliövaara and Väisänen (1993), Whittaker (1994), Docherty et al. (1997), and, most recently, Flückiger et al. (2002).

None of the studies reported in the past decade have clarified the situation in terms of clearly consistent effects. A 1997 review by Docherty et al. (1997), for example, examined 17 reports of studies of aphid species on a range of hosts and classed the O_3 effects on aphid performance as 35% positive, 41% negative, and 24% showing no significant effect. A tabulation of 19 studies by Flückiger et al. (2002) gave the corresponding figures: 42%, 21%, and 37%.

Other recent studies with the aphids *Schizolachnus pineti* and *Cinara pinea* on Scots pine (*Pinus sylvestris*) and *Cinara pilicornis* on Norway spruce *(Picea abies)* have also yielded variable results, but suggested that O_3 enhances aphid density on pine and aphid performance on spruce (Holopainen et al., 1997; Kainulainen et al., 2000a). In an earlier study with *Schizolachnus pineti* on Scots pine, Kainulainen et al. (1994) had observed no significant effects of O_3-treatment on aphid performance. However, more recent observations of long-term effects on aphid populations on aspen (*Populus tremuloides*) exposed to O_3 in a FACE system revealed that O_3 significantly increased aphid populations while decreasing the populations of predatory insects (Percy et al., 2002).

The observations of Brown et al. (1993) and Jackson (1995) led Whittaker (1994) and Brown (1995) to suggest that aphid response was dependent on ambient temperature as well as the dynamics of O_3 exposure and that growth tended to be stimulated with maximum temperatures below ~20 °C but was reduced at higher temperatures. The present situation with plant-aphid responses, therefore, remains confused and, although numerous suggestions have been offered to explain specific findings, they are difficult to assemble into a coherent picture.

Variability has also been found with the interactions involving chewing insects. For example, Lindroth et al. (1993) reported a small negative O_3 effect (8% reduction) on the growth of gypsy moth larvae (*Limantra dispar*) on hybrid poplar (*Populus tristis* × *P. balsamifera*) but no effect when growing on sugar maple (*Acer saccharum*). Ozone exposure reduced the growth rate of the larvae of the bug *Lygus rugulipennis* on Scots pine, but enhanced the growth of larvae of the sawfly *Gilpinia pallida* (Manninen et al., 2000). Costa et al. (2001) observed no significant O_3 effects on the growth and fecundity of the Colorado potato beetle (*Leptinotarsa decemlincata*) on potato (*Solanum tuberosum* L.) in greenhouse and field experiments.

Fortin et al. (1997), in a 2-year study of the forest tent caterpillar (*Malacosoma disstria*) on sugar maple, observed that O_3 exposure increased the growth rate of female larvae in only one year; fourth- and fifth-instar larvae also showed a feeding preference for treated foliage in that year. However, studies based on open-air exposures of aspen indicated O_3-enhanced growth of *M. disstria* in terms of pupal weight (Percy et al., 2002) and larval performance (Kopper and Lindroth, 2003b). Jackson et al. (2000) observed inconsistency in studies on the larva of the tobacco hornworm (*Manduca sexta*) on tobacco (*Nicotiana tabacum*). In one year, feeding on O_3-treated foliage resulted in significantly greater larval weight, whereas the increase was not statistically significant in a second year although survival was increased. Also, oviposition by hornworm moths was increased if ambient O_3 levels were increased by 70% and returned to normal in ambient O_3 levels (Jackson et al., 1999).

Studies of the two-spotted spider mite (*Tetranychus urticae*) on white clover (*Trifolium repens*) and peanut (*Arachis hypogeae*) by Heagle et al. (1994a) and Hummel et al. (1998) showed that O_3-exposure stimulated mite populations on an O_3-sensitive clover clone and on peanut. The lack of significant effects on mites on the O_3-resistant clover clone suggests that the responses were host-mediated.

There, therefore, appears to be a clearer indication of the likelihood that increased chewing insect and mite performance will result from O_3-induced changes in the host plant. However, negative effects continue to be reported, indicating that the response is probably also being determined, in part, by other environmental, genetic, or temporal variables.

Reported O_3-induced enhancement of attack by bark beetles (*Dendroctonus brevicomis*) on Ponderosa pine (*Pinus ponderosa*) has been suggested by Dahlsten et al. (1997) to be due to greater brood development on injured trees, possibly related to decreased numbers of predators and parasitoids. This view gains some support from the observation that O_3 exposure adversely affected the searching behavior of the parasitoid *Asobara tabida* for larvae of *Drosophila subobscura* which led to fewer parasitized fly larvae (Gate et al., 1995). Such observations reveal another level of complexity in the O_3-plant-insect interrelationship: O_3 may reduce the effectiveness of the natural control of insect pests. The phenomenon is probably related to effects on olfactory cues, as it was shown by Arndt (1995) that O_3 can affect fly behavior by modifying the pheromones involved in fly aggregation.

These reports focus on the direct or indirect effects on the insect or mite feeding on foliage previously or currently exposed to O_3. They provide little, if any, information on the host plant effects other than qualitative references to the injury caused by the O_3 exposure. Enhanced pest development will ultimately lead to increased adverse effects on the hosts in the long term, but the only report of an O_3-plant-insect interaction directly affecting the host plant in the short term still appears to be that of Rosen and Runeckles (1976). They found that infestation by the greenhouse whitefly (*Trialeurodes vaporariorum*) sensitized bean plants (*Phaseolus vulgaris*) to injury by otherwise noninjurious low levels of O_3, leading to premature senescence of the leaves.

The overall picture regarding possible O_3 effects on plant-insect relations, therefore, continues to be far from clear. Only a few of the very large number of such interactions that may affect crops, forest trees, and other natural vegetation have been studied. The trend suggested in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) that O_3 may enhance insect attack has received some support from a few recent studies. However, the variability noted in most of the studies makes it clear that we are still far from being able to predict the nature of any particular O_3-plant-insect interaction or its magnitude or severity.

AX9.3.3.2 Oxidant-Plant-Pathogen Interactions

Plant diseases are caused by pathogenic organisms, e.g., fungi, bacteria, mycoplasmas, viruses, and nematodes. Ozone impacts on disease are briefly discussed in earlier reviews by Ayres (1991) and Colls and Unsworth (1992) and, more recently, by Flückiger et al. (2002). Biotic interactions with forest trees have been reviewed by Chappelka and Samuelson (1998); Sandermann (1996) and Schraudner et al. (1996) have summarized molecular similarities and interrelationships between necrotic O_3 injury to leaves and pathogen attack. A few recent publications have added to our fragmented knowledge of O_3-plant-disease interactions and the mechanisms involved, but there appear to have been no reports to date of studies involving mycoplasmal diseases.

The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) noted the concept put forward by Dowding, (1988) "that pathogens and pests which can benefit from damaged host cells and from disordered transport mechanisms are enhanced by pollution insult to their hosts, whereas those pathogens and other symbionts which require a healthy mature host for successful invasion are depressed by pollutant stress to their host." The pathogens of the first type are mostly facultative necrotrophic fungal parasites, whereas the second type are largely obligate biotrophic fungi, bacteria, and viruses. Based on this distinction, the majority of the cases cited in the 1996 document supported Dowding's (1988) view, as have several more recent studies summarized in Table AX9-11. However, there are also some contradictions.

Most investigations have focused on the incidence and development of disease on plants previously or concurrently exposed to O_3, rather than on the corollary effect of disease on the response to O_3. In all of the studies of facultative pathogens and the nematode studies, exposure to O_3 tended to result in increased disease severity through increased spore germination or increased fungal growth and development; although in the case of grey mold (*Botrytis cinerea*) on kidney bean (*Phaseolus vulgaris*), this was only observed on an O_3-sensitive cultivar inoculated with conidia (Tonneijck, 1994). After mycelial inoculation, O_3 exposure reduced disease development in the O_3-sensitive cultivar, but no satisfactory explanation was offered to account for the difference in response. With poplar leaf spot, *Marssonina tremulae*, on hybrid poplar (*Populus trichocarpa* × *balsamifera*), low level exposures to O_3 increased disease (in agreement with theory) but higher levels (200 ppb, 8 h per day for 15 days) reduced conidial germination (Beare et al., 1999b).

Table AX9-11. Interactions Involving O_3 and Plant Pathogens

Host Plant	Pathogen	Effect of O_3 on Disease	Effect of Disease on O_3 Response	Reference
Obligate Biotrophs				
Bottle gourd (*Lagenaria siceraria*)	Powdery mildew (*Sphaerotheca fulginea*)	Increased in 50ppb O_3; decreased in 100+ppb	Decreased; partial protection	Khan and Khan (1998a)
Cucumber (*Cucumis sativa*)	Powdery mildew (*Sphaerotheca fulginea*;	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 (*Pisum sativum*)	Powdery mildew (*Erysiphe polygoni*)	Decreased infection	Decreased; partial protection	Rusch and Laurence (1993)
Aspen (*Populus tremuloides*)	Leaf rust (*Melampsora medusae* f. sp. *tremuloidae*)	Increased severity	Not reported	Karnosky et al. (2002)
Hybrid poplar (*Populus trichocarpa* × *balsamifera*)	Leaf rust (*Melampsora larici-populina* or *M. allii-populina*)	Increased infection and severity	Increased sensitivity (synergistic)	Beare et al. (1999a)
Broad bean (*Vicia faba*)	Bean rust (*Uromyces viciae-fabae*)	Not reported	Decreased; partial protection	Lorenzini et al. (1994)
Facultative Necrotrophs				
Kidney bean (*Phaseolus vulgaris*)	Grey mold (*Botrytis cinerea*)	Increased from conidia on O_3-sensitive cultivar; decreased from mycelium	Not reported	Tonneijck (1994)
	Grey mold (*Botrytis cinerea*)	Increased infection	Not reported	Tonneijck and Leone (1993)
	White mold (*Sclerotinia sclerotiorum*)	Increased infection	Not reported	Tonneijck and Leone (1993)
Scots pine (Pinus sylvestris)	Annosus root and butt rot *(Heterobasidion annosum*)	Increased development*	Not reported	Bonello et al. (1993)

Table AX9-11 (cont'd). Interactions Involving O_3 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 (*Pinus taeda*)	Pitch canker (*Fusarium subglutinans*)	Increased development	Increased sensitivity	Carey and Kelley (1994)
Hybrid poplar (*Populus deltoides* × *nigra*)	Canker (*Septoria musiva* [=*Mycosphaerella populinum*])	Increased incidence	Not reported	Woodbury et al. (1994)
Hybrid poplar (*Populus trichocarpa* × *balsamifera*)	Leaf spot (*Marssonina tremulae*)	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 (*Triticum aestivum*)	Blotch (*Septoria nodorum*)	Increased infection	Not reported	Tiedemann and Firsching (1993)
	Tan spot (*Pyrenophora tritici-repentis*)	Increased infection of disease-susceptible genotypes	Not reported	Sah et al. (1993)
Nematodes				
Tomato (*Lycopersicon esculentum*)	Root-knot nematode *(Meloidogyne incognita*)	Increased development	Increased foliar injury; reduced plant growth (synergistic)	Khan and Khan (1997); Khan and Khan (1998a)

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

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

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

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

Similarities between the sensitivities of different cultivars or clones to O_3 and to specific diseases have been noted. For example, Sah et al. (1993) found that the severity of injury caused by tar spot and O_3 exposures of 12 wheat (*Triticum aestivum* L.) cultivars were closely correlated ($R^2 = 0.986$). Such similarities appear to have a mechanistic basis, as several studies have noted similarities in the molecular and biochemical changes that occur in plants infected with pathogens and in O_3 exposed plants. Schraudner et al. (1992), Ernst et al. (1992), Eckey-Kaltenbach et al. (1994a,b), Yalpani et al. (1994), and Bahl et al. (1995) have presented evidence that O_3 exposures result in responses such as increased levels of salicylic acid, the signaling agent for increased induced resistance to pathogens. This, in turn, leads to the activation of the

genes that encode defense proteins, including the so-called pathogenesis-related proteins. The induction of such proteins might account for the decreased infection with *Sphaerotheca* and *Melampsora* at higher O_3 exposures but does not account for increased infections seen at lower exposure levels. The issue is discussed more fully by Sandermann (1996) and Schraudner et al. (1996). More recently Sandermann (2000) has extended the theory relating O_3 exposure and disease by suggesting that, because of O_3 "memory effects" in affected host plants that may persist over weeks or months, analysis for various induced biomarkers of gene activation may provide a useful tool for improving our ability to predict the outcome of O_3-plant-pathogen interactions.

There have been no reports of O_3 studies with mixed infections by pathogens, but the complete suppression of *Heterobasidion* butt and root rot of Scots pine by the mycorrhizal symbiont *Hebeloma crustuliniforme* indicates the possibility of interactions involving more than one fungus (see Section AX9.3.4.3.3 below).

In summary, our understanding of oxidant-plant-disease interactions is far from complete. However, a combined tabulation of the evidence presented in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) and that noted in Table AX9-11 leads to the following summary of O_3 effects on plant diseases and corollary effects of infection on plant response to O_3, as indicated by the number of studies showing increases or decreases in disease or susceptibility.

For obligate biotrophic fungi, bacteria, nematodes:

O_3 increased disease:	9	Increased susceptibility to O_3:	3
O_3 decreased disease:	15	Decreased susceptibility to O_3:	9

For facultative necrotrophic fungi:

O_3 increased disease:	25	Increased susceptibility to O_3:	2
O_3 decreased disease:	3	Decreased susceptibility to O_3:	4

Thus, although O_3 may reduce the severity, but not the incidence, of some of the diseases caused by the obligate pathogens, the evidence overall indicates that with most diseases, their severity is more likely to be increased by O_3 than not. However, the actual consequences will be specific to the disease and level of exposure, and, most importantly, will be determined by environmental suitability and epidemiological requirements for disease to develop. Conversely, some evidence

suggests that infection by obligate pathogens may confer some degree of "protection" against O_3, a dubious benefit from the plant's point of view.

AX9.3.3.3 Oxidant-Plant-Symbiont Interactions

No further studies have appeared regarding O_3 effects on the important bacterial symbiont of legumes, *Rhizobium*, since those summarized in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). Hence, our present understanding is that, although relatively high levels of exposure (>200 ppb) can result in severe (>40%) reductions in nodulation (and therefore nitrogen-fixation) on soybean roots, lower O_3 exposures may cause lesser reductions in nitrogen fixation. However, the data are inadequate to attempt to define any quantitative exposure-response relationships.

There have been a few recent reports on O_3-plant-mycorrhizae interrelationships. These have mostly involved seedlings of coniferous tree species. A transient O_3-induced stimulation of mycorrhiza on Scots pine roots reported by Kasurinen et al. (1999) was not observed in a later study by Kainulainen et al. (2000b). Studies of the mycorrhiza *Paxillus involutus* on birch (*Betula pendula*) seedlings showed that, although O_3 reduced mycorrhizal growth rate, it led to greater extension growth which in turn resulted in greater mycorrhizal infection of neighboring Aleppo pine (*Pinus halepensis*) seedlings (Kytöviita et al., 1999). However, O_3 reduced nitrogen acquisition by *P. halepensis* from its mycorrhizal symbiont (Kytöviita et al., 2001). The complex interrelationships that may occur in the rhizosphere were revealed by the observation by Bonello et al. (1993) that the mycorrhiza *Hebeloma crustuliniforme* could overcome the O_3-stimulated severity of root rot on Scots pine caused by the fungus *Heterobasidion annosum* (noted in Section AX9.3.4.3.2).

In summary, the available evidence is far too fragmented and contradictory to permit drawing any general conclusions about mycorrhizal impacts. The negative effects of O_3 on mycorrhizae and their functioning that have been reported have not necessarily been found to lead to deleterious effects on the growth of host plants. Thus, little has changed from 1991 when Dighton and Jansen (1991) asked: "Atmospheric Pollutants and Ectomycorrhizae: More Questions than Answers?". Because of their important roles in ecosystems, mycorrhizae are further discussed in Section AX9.6.

AX9.3.3.4 Oxidant-Plant-Plant Interactions: Competition

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

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

Although weak competition is the largest global cause of crop losses, little is known about the impact of O_3 on crop-weed interactions. The topic does not appear to have been investigated in recent years. We can only speculate as to the possible consequences of O_3 exposure on weed competition based on our limited understanding of the effects on a few, mostly two-component mixtures of cultivated species.

The tendency for O_3-exposure to shift the biomass of grass-legume mixtures in favor of grass species, reported in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) has been confirmed by recent studies. In a ryegrass (*Lolium perenne*) + clover (*Trifolium repens*) mixture grown in an open-air fumigation system, clover growth was impaired by extended exposures to above-ambient O_3, leaving patches for weed invasion (Wilbourn et al., 1995). An open-top chamber study (OTC) by Nussbaum et al. (1995b) using the same species confirmed the greater effect on clover but observed that the magnitude of the effect depended highly on the pattern of O_3-exposures over extended growing periods. Low-level exposures shifted species composition in favor of *Lolium*, but exposures to higher peak O_3 levels depressed total mixture yield. With an alfalfa (*Medicago sativa*) + timothy (*Phleum pratense*) mixture, Johnson et al. (1996a) noted that O_3 decreased alfalfa root growth and increased timothy shoot growth and height. Nussbaum et al. (2000a) reported that, with increased exposure to O_3, well-watered red clover (*Trifolium pratense*) plants suffered from increased competition from the grass *Trisetum flavescens*, but the O_3 exposure

also negatively affected grass growth, depressing overall total yield. However, a greater adverse effect on *Trisetum* resulted from O_3-induced increased competition when grown with brown knapweed (*Centaurea jacea*), a weed species.

Andersen et al. (2001) demonstrated the potential for competition and O_3 exposure to work together to affect the growth of tree seedlings. Ozone had no direct adverse effect on pine growth in a 3-year study of ponderosa pine (Andersen et al., 2001) seedlings grown in mesocosms with three densities of blue wild-rye grass (*Elymus glaucus*), but O_3 exposure increased the competitive pressure of the grass which caused a major reduction in pine growth.

Three studies have been reported on more complex plant associations. Ashmore and Ainsworth (1995) studied mixed plantings of two grasses, *Agrostis capilaris* and *Festuca rubra*, with two forbs[15], *Trifolium repens* (a legume) and *Veronica chamaedrys*, exposed to O_3 in OTCs. The proportion of forbs, *Trifolium* in particular, declined, especially when cut at biweekly intervals. In a related study, Ashmore et al. (1995) used artificial mixtures of grasses and forbs and transplanted swards of native calcareous grassland species and found that, regardless of whether total biomass was adversely affected by exposures to O_3, higher exposures progressively shifted species composition, usually at the expense of the forb species. The observed shifts in competitive balance in favor of grasses is consistent with observations that many grass species are less sensitive to O_3 than forbs. However, as previously shown by Evans and Ashmore (1992), knowledge of the relative sensitivities to O_3 of the component species grown in isolation or in monoculture does not always predict the impact of O_3 on the components in a mixed culture.

Barbo et al. (1998) exposed an early successional plant community to O_3 in OTCs for two growing seasons. Ozone decreased community structure features such as height of canopy, vertical canopy density (layers of foliage), and species diversity and evenness. Surprisingly, blackberry (*Rubus cuneifolius*), a species considered to be O_3-sensitive, replaced sumac (*Rhus copallina*) canopy dominance. Barbo et al. (2002) also demonstrated the role of competition in determining the impact of O_3 on loblolly pine (*Pinus taeda*). They reported that the increased growth of natural competitors in OTCs using charcoal-filtered air to reduce the ambient O_3 concentrations resulted in decreased pine growth. They noted that this is contrary to the

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

frequently reported increased growth observed in reduced O_3 levels in the absence of interspecific competition.

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

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

AX9.3.4 Physical Factors

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

Another physical feature of the microclimate, wind and air turbulence, which affects the thicknesses of the boundary layers over leaves and canopies and, hence, affects gas exchange

rates (including the fluxes of O_3 and other oxidants into the leaves) is discussed elsewhere (Section AX9.4).

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

AX9.3.4.1 Light

Plants are the primary producers of biomass on the planet through their ability to capture light energy (by the process of photosynthesis) and convert it to the many forms of chemical energy that sustain their own growth and that of secondary consumers and decomposers. Light *intensity* is critical because the availability of light energy (a resource, *sensu* Monteith and Elston (1993) governs the rate at which photosynthesis can occur, while light *duration* (i.e., photoperiod) profoundly effects development in many species. Although light *quality* (i.e., the distribution of incident wavelengths) may also affect some physiological plant processes, there is no evidence to indicate that such effects are of relevance to concerns over oxidant pollution, except at the short wavelengths of UV-B. This topic is discussed in the context of climate change in Section AX9.3.8.2, and as a stress factor per se affected by atmospheric O_3 in Chapter 10. However, as noted above and in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), none of these features is controllable in natural field situations. A brief discussion of light intensity-O_3 interactions is included in the review by Chappelka and Samuelson (1998).

The conclusion in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) that low light intensities and short photoperiods tended to increase susceptibility to foliar O_3-injury may still be valid, but this may or may not translate into adverse effects on growth. For example, Tjoelker et al. (1993) found that, when seedlings of sugar maple, a shade-tolerant species, were grown in 7% full sunlight, O_3 reduced shoot and root growth, but had no significant effect in 45% sunlight (a 6-fold increase). In contrast, the reverse was observed with a shade-intolerant hybrid poplar, with the greater impact of O_3 occurring in the higher light intensity treatment.

The greater sensitivity of maple in low light has also been confirmed in other studies. Tjoelker et al. (1995) noted a greater O_3-induced inhibition of photosynthetic CO_2 assimilation in shaded leaves than in leaves in full sunlight. However, in the absence of differences in stomatal conductance, the effect was considered to be independent of O_3 flux; it appeared to be a consequence of reduced chlorophyll contents and quantum efficiencies induced by O_3. In contrast, Bäck et al. (1999), who also observed a greater inhibition of net photosynthesis by O_3 in shaded leaves, reported decreased stomatal conductance. Although reduced conductance might suggest reduced O_3 flux and, therefore, decreased adverse effects, the authors concluded that the effects of reduced conductance were offset by long-term changes in leaf structure, leading to less densely packed mesophyll cells and greater internal air space within the leaves. Morphological differences between lower and upper crown leaves of black cherry (*Prunus serotina*) have been suggested as the basis for the greater O_3-susceptibility of the lower crown leaves (Fredericksen et al., 1995). Bäck et al. (1999) also observed accelerated foliar senescence induced by O_3 on shaded leaves, a response also noted by Topa et al. (2001). Sensitivity to O_3 was found to be increased in shade- but not sun-leaves of shade-tolerant red oak (*Quercus rubra*) (Samuelson and Edwards, 1993). Similarly, Mortensen (1999) observed that seedlings of mountain birch (*Betula pubescens*) grown in 50% shade suffered greater foliar injury from O_3 than those grown in full sunlight.

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

The suggestion of greater sensitivity to O_3 of shade-tolerant species in low-light conditions and the greater sensitivity of shade-intolerant species in high light is somewhat of an oversimplification when dealing with mature trees, for which light intensity varies considerably within the canopy because of shading. Chappelka and Samuelson (1998) noted that the

interaction between sensitivity to O_3 and the light environment in forest trees is further complicated by developmental stage, with seedlings, saplings, and mature trees frequently giving different results. Topa et al. (2001) also cautioned that O_3 effects on leaf-level photosynthesis may be poor predictors of the growth responses of sugar maple in different light environments.

In high-light intensities, many species exhibit some degree of photoinhibition of the photosynthetic process through the overloading of the mechanisms that protect the photosynthetic reaction centers in the chloroplasts. Guidi et al. (2000) reported complex interactions between high-light intensities (inducing photoinhibition) and O_3 exposures in kidney bean with high intensities tending to enhance the detrimental effect of O_3 on photosynthesis. One of the studies in the extensive European Stress Physiology and Climate Experiment-wheat (ESPACE-wheat) program (Bender et al., 1999), conducted in 1994 and 1995, included an investigation of the effects of climatic variables on yield response to O_3 using two simulation models, AFRCWHEAT2-O_3 and LINTULCC (Ewart et al., 1999; Van Oijen and Ewart, 1999). Among the observed trends, it was noted that relative yield loss of wheat due to elevated O_3 tended to increase with light intensity. In contrast, Balls et al. (1996) used ANNS to investigate microclimatic influences on injury caused by O_3 to clover (*Trifolium subterraneum*) and found that, especially at mid-range cumulative O_3 exposures (350 to 500 ppb-h), injury tended to decrease with increasing light intensity. Similar observations by Davison et al. (2003) of foliar injury to wild populations of cutleaf cone flower (*Rudbeckia laciniata*) exhibiting a range of PAR levels within their canopies led the authors to conclude that the variation in injury symptoms observed was "unlikely to be due to differences in ozone flux and more likely to be due to variation in light." Antonielli et al. (1997) found evidence indicating that the high sensitivity of the bioindicator tobacco cultivar *Nicotiana tabacum* cv. Bel-W3 is partly determined by its high photosynthetic electron transport rates at high-light intensities, which exceed the capabilities of the plant to dissipate energy and oxyradicals.

The 1996 O_3 AQCD referred to the important role of light in controlling stomatal opening and suggested that light duration (i.e., photoperiod) might dictate the actual uptake of O_3 to some degree. However, it should also be noted that Sild et al. (1999) found that clover plants could suffer foliar injury even if they were exposed to O_3 during the dark period of the day-night cycle, when stomatal conductance is at its lowest.

A possible indirect effect of light intensity was noted by Reiling and Davison (1992c) in their study of the O_3-tolerance of common plantain (*Plantago major* L.) plants grown from seeds collected from populations at 28 different sites in Britain. Ozone-tolerance, defined in terms of plant growth, was found to be a function of both previous O_3-exposure history and hours of bright sunshine during the year before the seeds were collected. However, the authors cautioned that, since tropospheric O_3-formation is itself dependent upon irradiation, the observation does not necessarily imply a direct effect of light intensity on the plants' response to O_3.

The only recent studies concerning interactions with light quality appear to be those involving O_3 and UV-B as a component of climate change. These are dealt with in Section AX9.3.4.8.2. The effects of photoperiod on response to O_3 or the converse do not appear to have received any recent attention.

Although the intensity, quality, and duration of light are not controllable in the natural world, the interactions of O_3 with light intensity, in particular, clearly have relevance to the growth of shade-tolerant and shade-intolerant species in mixed forest stands. It appears that the nature of light intensity-O_3 interactions may depend upon the type of light environment to which the species are best adapted, with increased light intensity increasing the sensitivity of light-tolerant and decreasing the sensitivity of shade-tolerant species to O_3. Although there is certainly some evidence to the contrary, this hypothesis is a reasonable summation of current understanding with regard to O_3-light intensity interactions.

AX9.3.4.2 Temperature

"Temperature determines the start and finish and rate and duration of organ growth and development" (Lawlor, 1998). Such processes depend on fundamental physiological activities that are mostly enzyme-mediated and whose kinetics are directly affected by temperature. Since the processes of enzyme deactivation and protein denaturation also increase as temperatures rise, each enzymatic process has a unique optimum temperature range for maximal function. However, the optima for different processes within the plant vary appreciably and, hence, the optimum temperature range for overall plant growth is one within which all of the individual reactions and vital processes are *collectively functioning optimally,* not necessarily *maximally*. Furthermore, individual features of plant development (e.g., shoot and root growth, flowering, pollen tube growth, fruit set, seed development) have different specific optima, so that

differential responses to temperature occur, leading to temperature-induced developmental changes. For example, despite increased assimilation, increased temperatures may result in decreased grain yields of crops such as wheat, because the growing season is effectively shortened by a more rapid onset of senescence (Van Oijen and Ewart, 1999).

Rowland-Bamford (2000) noted that a plant's response to temperature changes will depend upon whether it is growing at its near optimum temperature for growth or its near maximum temperature and whether any increase in mean temperature results in temperatures rising above the threshold for beneficial responses. Impairment by O_3 of any process may be thought of as being analogous to a downward shift below and away from the temperature optimum or an upward shift above and away from the optimum. Since a temperature rise toward the optimum would result in a rate increase, the combined effects of O_3 and such an increase might neutralize each other, while the effects of O_3 and a decrease in temperature would likely be additively negative. Above the optimum temperature, the situations would be reversed with the effects of increased temperatures and O_3 being additively negative, and decreasing temperatures counteracting any negative effect of O_3. Thus, it is difficult to generalize about the interactions of temperature and O_3 on overall plant responses such as growth in which the different temperature-rate relationships of different growth components are merged, because they depend upon the relationship of any temperature changes to the optimum for a species.

Studies of the effects of temperature on the impact of O_3 have increased recently because of an increased need to understand the consequences of global warming as a component of climate change. Direct interactions of temperature with O_3 are reviewed here, but the issues are addressed again in Section AX9.3.8.1 in relation to changes in atmospheric CO_2 levels.

The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) stressed the interdependence of the temperature within the tissues of the leaf (where the various temperature-sensitive processes occur) on three distinct components: the ambient air temperature, the heating effect of incident infrared radiation during the photoperiod, and the evaporative cooling effect caused by transpirational loss of water. It also cautioned that, especially in experiments using controlled environment chambers, the effects of temperature could well be confounded with those of humidity/vapor pressure deficit (VPD). Temperature and VPD are strongly interrelated, and VPD plays an important role in regulating stomatal transpiration. Because of the role that evaporative cooling plays in determining internal leaf temperatures, any factor that causes

stomatal closure and reduced conductance inevitably leads to increased leaf temperatures. Such interactions add to the difficulties in distinguishing the effects of temperature from those of other factors, as actual leaf temperatures are rarely measured and reported.

Despite these caveats, there is some evidence that temperature per se influences plant response to O_3. For example, in rapid-cycling Brassica (*Brassica rapa*) and radish (*Raphanus sativus*), marked O_3-inhibitions of growth were observed at low root temperatures (13 °C) but not at 18 °C (Kleier et al., 1998, 2001). With regard to air temperature, this was included in the range of micrometeorological variables studied in several recent extensive field studies and was found to have a significant effect on response to O_3 in most cases. Balls et al. (1996) used ANNs in an analysis of the growth of clover (*Trifolium subterraneum*) and concluded that light and VPD had greater influences than temperature on the visible injury response to O_3. However, in three studies with different cultivars of white clover (*Trifolium repens*), temperature was found to be important to the growth response. Ball et al. (1998) exposed *T. repens* cv. Menna to ambient O_3 in OTCs at 12 European sites at a range of latitudes and altitudes from 1994 to 1996. The impact of O_3 on growth was determined as the ratio of growth with and without treatment with the O_3-protectant, EDU (see Section AX9.2). Artificial neural network analysis showed that O_3 exposure (measured as the AOT40 index, see Section AX9.3.6), VPD, and temperature were consistently the three most important variables governing response to O_3 over a range of different ANN models. However, the authors did not describe the form of the O_3-response relationship with temperature. Similar observations were reported by Ball et al. (2000) and Mills et al. (2000) for O_3-sensitive and -tolerant clones of *T. repens* cv. Regal, grown at 14 to 18 European locations from 1995 to 1998. In both studies, the impact of O_3 was measured as the sensitive/tolerant growth (biomass) ratio. Although Ball et al. (2000) found temperature to be less important than O_3 exposure and VPD, Mills et al. (2000) found temperature to be the most important input variable after O_3 exposure (AOT40). In both cases, the adverse effect of O_3 increased with increasing temperature.

A study of black cherry seedlings and mature trees in Pennsylvania, using micrometeorological variables aimed to predict O_3 uptake, found temperature to be unimportant (Fredericksen et al., 1996b), but in the study of populations of common plantain referred to in Section AX9.3.4.1, Reiling and Davison (1992c) noted a weak, positive correlation between mean temperature at the collection site and O_3 tolerance (based on growth rate) of the different

populations. In contrast, Danielsson et al. (1999) collected genotypes of *Phleum arvense* from a wide range of Nordic locations and found a positive effect of temperature on the growth of genotypes from locations with higher summer temperatures, but sensitivity to O_3 did not vary systematically with geographic location.

Van Oijen and Ewart (1999) studied the effects of climatic variables on the response to O_3 in the ESPACE-wheat program, based on two distinctive simulation models (AFRCWHEAT2-O_3 and LINTULCC [Ewart et al., 1999]) and noted that although the relative yield loss of wheat due to elevated O_3 tended to increase with temperature, the effect was of minor significance.

In contrast to the variable results obtained in studies of the effects of temperature on response to O_3, the corollary effect of O_3 exposure on subsequent sensitivity to low temperature stress, noted in the 1996 criteria document, is well recognized. In reviewing low temperature-O_3 interactions, Colls and Unsworth (1992) noted that winter conditions produce three kinds of stress: desiccation, chilling or freezing temperatures, and photooxidation of pigments. Of these, they suggested that while the first two were important, the last may play a particularly significant role because the "combination of high irradiance and low temperatures permits a buildup of free radicals in leaf tissue, and these free radicals then attack chlorophyll." Chappelka and Freer-Smith (1995) suggested that the injury and losses to trees caused by this delayed impact of O_3 may be equally or more important than the direct impacts of O_3 on foliage of visible injury and necrosis, or the disruption of key physiological processes such as photosynthesis. In this context, the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) referred to the conceptual framework of Eamus and Murray (1991), which is still valid: brief periods of mild temperatures in the severest winters result in dehardening; O_3 decreases frost hardiness per se, but also increases the predisposition to dehardening; dehardening places O_3-exposed trees at greater risk from subsequent low temperatures. However, no quantified models of these effects have yet appeared.

The 1996 O_3 AQCD also noted that O_3 adversely affects cold hardiness of herbaceous species. More recently, Foot et al. (1996, 1997) observed winter injury and decreased growth in low-growing perennial heather *Calluna vulgaris* exposed to O_3 (70 ppb, 8 h/day, 5 days/week for 6 months) during the winter (6.8 °C mean), but found no significant effects from the same exposures during the summer (12.3 °C mean). Although Potter et al. (1996) observed a similar

situation with the moss *Polytrichum commune*, the reverse was found with the moss *Sphagnum recurvum*.

In summary, unequivocal evidence exists that O_3 causes sensitization to the adverse effects of low temperatures, but there is no clear pattern in the evidence regarding the effects of temperature on O_3 response. The many contradictory responses to temperature and O_3 probably reflect our lack of detailed knowledge of the temperature optima for the different growth components of the studied species. The topic of temperature-oxidant interactions is revisited later in Section AX9.3.4.8 in the context of global warming as a feature of climate change.

AX9.3.4.3 Humidity and Surface Wetness

The moisture content of the ambient air (or its VPD) is a rate modifier (*sensu* Monteith and Elston (1993)) and an environmental regulator of stomatal conductance. Both of the previous O_3 AQCDs (U.S. Environmental Protection Agency, 1986, 1996) concluded that the weight of evidence indicated that high RH (=low VPD) tended to increase the adverse effects of O_3, principally because the stomatal closure induced in most situations by O_3 is inhibited by high RH, leading to increased O_3 flux into the leaves.

Recent reports have confirmed this role of RH. The studies by Balls et al. (1995, 1996) and Ball et al. (1998) showed that VPD was an important determinant of O_3-induced injury and reduced growth in two species of clover, *Trifolium repens* cv. Menna and *T. subterraneum*. However, Mills et al. (2000) found it to be unimportant in the case of *T. repens* cv. Regal. Such difference between cultivars is not unexpected , because considerable differences also occur among species and genera. For example, Bungener et al. (1999a) studied 26 Swiss grassland species and found clear evidence that O_3 injury increased with decreased VPD (i.e., increased RH) in only eight species. However, the 1995 data from the European cooperative study of O_3 injury, which involved 28 sites in 15 countries and six crop species, led to the development of two 5-day critical-level scenarios involving O_3-exposure (calculated as the AOT40 index) and mean VPD (0930-1630h): 200 ppb-h at >1.5 kPa, and 500 ppb-h at < 0.6 kPa (Benton et al., 2000).

With forest tree species, Fredericksen et al. (1996b) found significant correlations between stomatal conductance of black cherry leaves and RH (+ve) and VPD (−ve), and studies on free-standing Norway spruce and larch (*Larix decidua*) showed that although ambient VPD was

highly positively correlated with ambient O_3 concentration, increased VPD caused stomatal closure, reducing O_3 uptake and impact (Wieser and Havranek, 1993, 1995).

Surface wetness may affect O_3 response through its direct effects on deposition to the surface and through changes in RH. Effects on the deposition of O_3 have been reviewed by Cape (1996). A surface film of water on leaves was found to increase O_3 deposition in four studies involving field-grown grape (*Vitis vinifera*) (Grantz et al., 1995), red maple (*Acer rubrum*) (Fuentes and Gillespie, 1992), deciduous forest dominated by largetooth aspen (*Populus grandidentata*) and red maple (Fuentes et al., 1992), and clover-grass mixed pasture (*Trifolium pratense*, *Phleum pratense*, and *Festuca pratensis*) (Pleijel et al., 1995a). In each case, the increased deposition could be attributed partly to an increased stomatal conductance through the abaxial (lower) surface and partly to uptake into the aqueous film on the adaxial (upper) surface. In contrast, decreased deposition was noted by Grantz et al. (1997) with field-grown cotton (*Gossypium hirsutum*). Since cotton is amphistomatous, with functional stomata on both leaf surfaces, it was suggested that, in this case, the water layer effectively sealed the adaxial surface stomata, more than offsetting any increase in conductivity of the stomata in the abaxial surface. However, none of the studies investigated the consequences of the differences in deposition. Although it could be inferred that, with part of any increased deposition being the result of increased O_3 flux into the leaves, there would be the likelihood of increased O_3 adverse effects, as suggested by earlier studies (Elkiey and Ormrod, 1981) that, by misting bluegrass (*Poa pratensis*) during exposure to O_3, injury was significantly increased.

To conclude, the effects of high RH (low VPD) and surface wetness have much in common, as they both tend to enhance the uptake of O_3, largely through effects on stomata leading to increased impact.

AX9.3.4.4 Drought and Salinity

The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) concluded that the available evidence clearly indicated that exposure to drought conditions could reduce the adverse effects of O_3 on the growth of herbaceous and woody plants, but it also noted that no quantitative models of the O_3-soil moisture deficit (SMD) interaction had yet appeared in print. Nevertheless, the "protective" effect was inconsistent, and only appeared when SMD was accompanied by high evaporative demand. Since that time, further studies have confirmed the

interaction, and simulation models have begun to appear. Mills (2002) has recently provided a brief review of the topic.

With regard to herbaceous species, Vozzo et al. (1995) observed less O_3-induced injury and suppression of net photosynthesis and growth in water-deficient soybean (*Glycine max*) than in well-watered plants. In several studies with wheat (*Triticum aestivum*), on the other hand, although adverse effects of both O_3 and SMD were noted, they were consistently additive (Bender et al., 1999; Fangmeier et al., 1994a,b; Ommen et al., 1999).

In attempting to model the stomatal conductance of wheat in relation to O_3 and soil moisture, Grüters et al. (1995) found that although O_3-induced stomatal closure was enhanced by SMD, reducing O_3 uptake, the R^2 of the overall model was only 0.40, indicating that other significant factors or relationships were involved.

With regard to native vegetation, Bungener et al. (1999a) used mixed plantings of 24 Swiss grasses, herbs, and legumes and observed that, although O_3-drought interactions were species-specific, they tended to reflect stomatal functioning. They found that SMD reduced O_3 injury in two clovers (*Trifolium repens* and *T. pratensis*) and two grasses (*Trisetum flavescens* and *Bromus erectus*), but noted no interactions in the other 20 species. With relative growth rate as the measure of response to O_3, interactions with SMD were noted in only three species: *Trifolium repens* and two weedy herbs, *Knautia arvense* and *Plantago lanceolata* (Bungener et al., 1999b). Although this variability in response among species was noted in the review by Davison and Barnes (1998), they also pointed out that in severely droughted regions of Europe, notably in Greece and Spain, O_3-induced injury and growth reductions were common on many (usually irrigated) crops, but there were virtually no records of injury symptoms in wild species.

Thus, the situation with herbaceous species is essentially unchanged from 1988 when Heagle et al. (1988) summarized the extensive NCLAN experiments that incorporated water stress as a variable: "SMD can reduce the response of crops to O_3 under some conditions but not under other conditions. Probably the occurrence of O_3 by SMD interactions was dependent on the degree of SMD-induced plant moisture stress."

With regard to trees, O_3 interactions with soil water availability have been discussed in several recent reviews: Chappelka and Freer-Smith (1995), who focused on O_3-induced predisposition to drought stress (Johnson et al., 1996b; Chappelka and Samuelson, 1998; and Skärby et al., 1998).

Several recent studies with conifers have yielded mixed results. No interactions with drought were observed by Broadmeadow and Jackson (2000) on Scots pine, by Karlsson et al. (2002) on Norway spruce, or by Pelloux et al. (2001) on Aleppo pine. More recently, Le Thiec and Manninen (2003) reported that drought reduced O_3-induced growth suppression of Aleppo pine seedlings. Panek and Goldstein (2001) inferred less impact of O_3 on droughted Ponderosa pine, and Van Den Driessche and Langebartels (1994) reported that drought reduced injury and O_3-induced ethylene release by Norway spruce. But Karlsson et al. (1997), in a comparative study of fast- and slow-growing clones of *P. abies*, only observed a drought-induced reduction of O_3-inhibited root growth in the fast-growing clone. In contrast, Grulke et al. (2002b) noted a synergistic interaction between O_3 and drought stress on gross photosynthesis of *Pinus ponderosa*, and Wallin et al. (2002) reported a synergistic growth response of Norway spruce in the third year of a 4-year study. A similar response was noted by Dixon et al. (1998) with the Istebna strain of Norway spruce.

With broad-leaved trees, studies of Durmast oak (*Quercus petraea*) (Broadmeadow et al., 1999; Broadmeadow and Jackson, 2000) and European ash (*Fraxinus excelsior*) (Broadmeadow and Jackson, 2000; Reiner et al., 1996) showed that drought provided partial protection against O_3-induced growth reduction. Although European beech (*Fagus sylvatica*) is reportedly an O_3- and drought-sensitive species, neither Pearson and Mansfield (1994) nor Broadmeadow et al. (1999) observed any interactions between these stresses, while Dixon et al. (1998) observed partial protection. Pääkönen et al. (1998) observed only additive effects in a sensitive clone of birch (*Betula pendula*). However, the experiments of Schaub et al. (2003) and the survey by Vollenweider et al. (2003a) on black cherry clearly indicate antagonism between drought and O_3 stresses on this species.

With regard to the converse effect, in a critical review of the evidence for predisposition to drought stress being caused by O_3, Maier-Maercker (1998) supported the hypothesis and suggested that the effects were caused by the direct effects of O_3 on the walls of the stomatal guard and subsidiary cells in the leaf epidermis, leading to stomatal dysfunction.

The Plant Growth Stress Model (PGSW) developed by Chen et al. (1994) is a physiology-based process model which includes drought among several environmental variables. Simulations for Ponderosa pine incorporated antagonistic effects between O_3 and drought stresses, i.e., partial protection, although Karlsson et al. (2000) have since emphasized that

drought-induced "memory effects" should be considered when developing simulation models incorporating stomatal conductance.

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

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

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

In summary, the recently described interactions of O_3 and drought/salinity stresses are consistent with the view that, in many species, drought/salinity reduces the impact of O_3, but O_3 increases sensitivity to drought stress, i.e., the type of response is determined by the sequence of stresses. However, synergisms have also been observed and any antagonisms are species-

specific and unpredictable in the absence of experimental evidence. In no case has an antagonism been found to provide complete protection.

AX9.3.5 Nutritional Factors

The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) noted that the large number of macro- and micronutrients and the wide range of species had limited the number of experimental investigation to all but a few cases of nutrient-O_3 interactions and most of these concerned nitrogen (N) and crops or forest tree species. The document also provided a comprehensive tabulation of the results of the relevant studies up to 1992.

The suboptimal supply of mineral nutrients to plants leads to various types of growth reductions. The consequences of suboptimal nutrition might, therefore, be expected to have some similarities to those of O_3 exposure. One might expect nutritional levels below the optimum either to amplify any effects of O_3 or at least lead to additive responses. The difficulty with this suggestion is that the available information has mostly been obtained from experimentation conducted using two or more arbitrarily selected levels of fertility with little or no regard to optima. Hence, it is not surprising that there have been contradictory reports, even among studies with the same species or cultivars conducted by different workers at different locations using different soils or soil mixes.

There appear to have been no recent studies on O_3 interactions with specific mineral nutrients other than N. Hence, the previous conclusions are still valid, viz. that increasing levels of the major elements potassium (K) and sulfur (S) usually reduce the impact of O_3, or, deficiency increases susceptibility, whereas increased phosphorus (P) usually increases injury, or, deficiency decreases susceptibility.

However, with N, a relationship to the optimum is usually demonstrable. Several earlier studies of $O_3 \times$ N interactions reported that the adverse effects of O_3 on growth were greatest at the optimum and decreased with increasing N-deficiency, a finding supported by the work on aspen of Pell et al. (1995), who also confirmed that excess N decreased O_3 impact on growth. Similarly, the adverse effects of O_3 on growth rate in wheat diminished with decreased N supply (Cardoso-Vilhena and Barnes, 2001). However, the effects of N are far from consistent. For example, Greitner et al. (1994) reported that O_3 and N-deficiency acted additively in aspen in reducing leaf surface area and rate of photosynthesis, Bielenberg et al. (2001) reported that the

rate of O_3-induced senescence was increased by N-deficiency in hybrid poplar (*Populus trichocarpa* × *P. maximovizii*), and Pääkkönen and Holopainen (1995) observed the least adverse effects of O_3 on European white birch (*Betula pendula*) at optimum N-fertility levels. With cotton, increased N-levels more than overcame the adverse effect of O_3 on growth and boll yield (Heagle et al., 1999b). In view of these contradictions, one may conclude that other, unrecorded factors may have contributed to the various findings. Thus, much remains unclear about O_3 × N-fertility interactions.

There have been two recent studies on the effects of overall soil fertility. Whitfield et al. (1998) observed that low general fertility increased O_3 sensitivity in selections of common plantain. At the biochemical level, well-fertilized European white birch saplings were found to be less adversely affected by O_3 than nutrient-stressed plants (Landolt et al., 1997).

TREEGRO model simulations of the growth of red spruce (*Picea rubra*) in conditions of nutrient deficiency and O_3 stress showed that, in combination, the two stresses acted less than additively (Weinstein and Yanai, 1994). Minimal amelioration by nutrient deficiency was predicted with Ponderosa pine.

Plants may also obtain N and S from airborne sources such as NO_x, HNO_3, NO_3^-, SO_2, and SO_4^{2-}, although, depending upon their concentration, these may also be phytotoxic. In various parts of the world, the deposition of N and S in these forms contributes significantly to the levels of nutritionally available N and S in soils. Such depositions may, in turn, influence the impact of O_3 on sensitive species through their roles as nutrients independent of any interactions that may occur because of their acidic properties (see Section AX9.3.6.5). For example, Takemoto et al. (2001) recently reviewed the situation in southern California's mixed conifer forests and noted that, where N-deposition is appreciable, its combination with O_3 is causing a shift in Ponderosa pine biomass allocation toward that of deciduous trees, with increased needle drop so that only 1- and 2-year needle classes overwinter. Such changes are having significant consequences on the balance of the forest ecosystem and are discussed more fully in Section AX9.5.

Of the micronutrient elements, only manganese (Mn) appears to have been studied recently. In beans (*Phaseolus vulgaris*) Mn-deficiency increased O_3 toxicity, despite causing reduced O_3 uptake (through decreased stomatal conductance) and inducing increased levels of Mn-SOD (Mehlhorn and Wenzel, 1996).

In view of the foregoing, it is impossible to generalize about the interactions of soil fertility with O_3. While this is especially true of the interactions involving soil nitrogen, for which there is much conflicting evidence, the interactions with other nutrients need much more thorough investigation than has occurred to date, before any clear patterns become apparent.

AX9.3.6 Interactions with Other Pollutants

The ambient air may have pollutant gases other than O_3 and its photochemical oxidant relatives. In particular, industrial, domestic, and automobile emissions and accidents can lead to significant atmospheric concentrations of gases such as sulfur dioxide (SO_2) and nitric oxide (NO) and nitrogen dioxide (NO_2), collectively referred to as NO_x, both locally and regionally. Local releases of gases such as hydrogen fluoride (HF), hydrogen chloride (HCl), and chlorine (Cl_2) may result from industrial emissions and accidents. Agricultural fertilizer and manure usage can lead to significant increases in ambient ammonia (NH_3) and ammonium sulfate ($(NH_4)_2SO_4$). The sulfur and nitrogen oxides may undergo reactions in the atmosphere leading to the formation of sulphate (SO_4^{2-}) and nitrate (NO_3^-) ions and resultant acid deposition.

The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) discounted much of the early research on pollutant combinations, because of its lack of resemblance to the ambient experience: the concentrations used were unrealistically high or the exposure regimes employed almost invariably used gas mixtures, whereas Lefohn et al. (1987) showed that the co-occurrence patterns of significant levels of O_3 with SO_2 or NO_2 in the United States were most frequently sequential or partially sequential with overlap; only rarely were they entirely concurrent. On the other hand, O_3 and peroxyacetylnitrate (PAN) frequently co-occur, as both form photochemically under similar conditions.

To the list of reviews mentioned in the 1996 O_3 AQCD should be added the more recent ones by Barnes and Wellburn (1998), Robinson et al. (1998), and Fangmeier et al. (2002), which also explore some of the potential mechanisms underlying pollutant-pollutant interactions.

AX9.3.6.1 Oxidant Mixtures

In 1998, Barnes and Wellburn noted that virtually no information existed on the effects on plants of concurrent exposures to O_3 and other components of photochemical oxidant other than PAN. The situation has not changed since their review appeared, and the topic appears to have

attracted no research interest since before the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). The continuing conclusion must, therefore, be that, from the limited information available, the two gases appear to act antagonistically, with O_3 raising the threshold for the visible injury response to PAN and PAN reducing the harmful effects of O_3.

AX9.3.6.2 Sulfur Dioxide

In reviewing $O_3 \times SO_2$ interactions, Barnes and Wellburn (1998) remarked: "The outcome of exposure to this combination of pollutants has probably been the most studied, yet is one of the least understood." More recent studies have only added to the conflicts referred to in the 1996 criteria document (U.S. Environmental Protection Agency, 1996), rather than resolve them. For example, Diaz et al. (1996) reported that, after a year of daily exposures of Aleppo pine seedlings to 50 ppb O_3 and/or 40 ppb SO_2, the combination of pollutants synergistically reduced shoot and root growth and impaired mycorrhizal colonization of the roots. With tomato (*Lycopersicon esculentum*), on the other hand, effects on growth ranged from synergistic at low exposures (50 ppb) to antagonistic at exposures of 200 ppb of each gas (Khan and Khan, 1994). Although various physiological measurements were made in these and earlier studies, it has not been possible to determine any consistent mechanism or mechanisms that might account for the conflicting results.

Since the information available about $O_3 \times SO_2$ interactions appears to be highly dependent upon species, the type of response measured, and the experimental protocol used, it would still appear prudent to heed the statement of Heagle et al. (1988) in their summary of the studies undertaken in 12 field experiments over several years within the NCLAN program: "There were no cases where O_3 and SO_2 interactions *significantly* affected yield." (emphasis added.)

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

The major oxides of nitrogen that occur in ambient air are nitrous oxide (N_2O), NO, and NO_2, of which the latter two (conveniently symbolized as NO_x) are particularly important in connection with O_3, because they are components of the reaction mix that leads to photochemical O_3 formation and because they can interact with O_3-responses. Their reactions in the atmosphere can also lead to the occurrence of nitric acid vapor (HNO_3) in ambient air. The other major

N-containing contaminant of ambient air in many parts of the world is NH_3, largely released through agricultural practices.

Despite various combinations of O_3 and NO_x being probably the most common air pollutant combinations found in the field, Barnes and Wellburn (1998) noted that they have been little studied. Much early work with O_3 and NO_x focused on $O_3 \times NO_2$ interactions and can be discounted, because of the unrealistic concentrations employed and their use as mixtures rather than in types of sequences. The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) concluded that evidence from studies involving concurrent exposures to both O_3 and NO_x at realistic concentrations was so fragmented and varied that no firm conclusions could be drawn as to the likelihood and nature such interactions. However, the few recent investigations taken together with the earlier data are now beginning to reveal a pattern of response.

With regard to NO, Nussbaum et al. (1995a, 2000b) reported their findings with concurrent exposures to NO and O_3 and observed that, at low O_3 levels, NO tended to act similarly to O_3 by increasing the scale of responses such as growth reductions. However, in ambient air in which O_3 is a dominant factor, the effects of NO were usually found to be negligible due to low levels, although the authors admitted that the effects observed were confounded by the inevitable O_3-induced oxidation of NO to NO_2.

Two possible mechanisms whereby NO may influence plant response to O_3 are suggested by recent biochemical studies. First, there is growing evidence for the role of NO as a signaling agent in plants that can induce defense responses to a range of biotic and abiotic stressors (Beligni and Lamattina, 2001; Neill et al., 2002). Second, a role for NO as an antioxidant scavenger of reactive oxygen species has been demonstrated by Beligni and Lamattina (2002) in potato leaves and chloroplasts. However, both of these cases concern endogenously synthesized NO, and it must be noted that in none of these or other reports of studies of NO signaling have the authors considered the potential significance of exogenous NO in ambient air.

An independent case for $O_3 \times NO$ interactions comes from Mills et al. (2000). The ANN model developed to predict the O_3 effects on white clover biomass based on experiments at 18 locations throughout Europe suggested that the minimum daily NO concentration (at 5 p.m.) may have been a contributor to adverse effects.

Turning to NO_2, Maggs and Ashmore (1998) found that, although concurrent but intermittent exposures of Bismati rice (*Oryza sativa*) revealed no significant growth

interactions, NO_2 reduced the rate of O_3-induced senescence, an antagonistic response possibly related to enhanced N-metabolism.

With regard to sequential exposures, two studies on gene activation in tobacco revealed that NO_2 counteracted the effect of O_3 in reducing mRNA levels for three genes encoding photosynthetic proteins (Bahl and Kahl, 1995) and tended to counteract the O_3-induced enhancement of defense-protein gene activation (Bahl et al., 1995). However, despite compelling evidence for significant interactive effects provided by earlier studies (Bender et al., 1991; Goodyear and Ormrod, 1988; Runeckles and Palmer, 1987), the only recent investigation of growth effects seems to have been that of Mazarura (1997) using sine-wave exposure profiles. He found that although 4 weeks of twice daily 3-h exposures to NO_2 (120 ppb peak concentrations) slightly stimulated growth of radish (*Raphanus sativa*) and while daily 6-h exposures to O_3 (120 ppb peak concentration) did not significantly reduce growth, the daily sequence, NO_2 - O_3 - NO_2, led to a 13% drop in dry matter production.

The combined evidence to date, therefore, suggests that, in leguminous species, the effects of these sequences are antagonistic with NO_2 tending to reduce (or reverse) the negative effects of O_3 on growth, while the effects are increased in other species. These conclusions differ from those of Barnes and Wellburn (1998) who suggested that sequential exposures tended to result in antagonistic effects (largely based on the summary by Bender and Weigel (1992), whereas simultaneous exposures were likely to lead to synergistic responses. With disagreements both among the data and their interpretation, it is not possible to determine the circumstances under which specific interactions of O_3 and NO_2 may occur, but there is no reason to doubt the validity of the individual findings of each study. Far more systematic investigation is needed to clarify the situation.

There appear to have been no studies of O_3 interacting with HNO_3 in the vapor phase. However, in the southern California montane forests (Takemoto et al., 2001), in Sweden (Janson and Granat, 1999), and elsewhere, significant amounts of N are deposited in this form because of the vapor's high deposition velocity. As a consequence, although much of it ultimately reaches the ground through leaching and leaf fall and enters the soil as NO_3^-, it may also be used as a N source by the foliage itself (Garten and Hanson, 1990; Hanson and Garten, 1992; Norby et al., 1989). This nutritional role is independent of any contribution that HNO_3 vapor may make to acidic deposition. Indirect interactions with the effects of O_3 through N-deposition of NO_x,

HNO_3, and NH_3 are related to the interactions of O_3 with N as a nutrient, and have recently been examined in the review by Takemoto et al. (2001). The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) stated that the evidence available at that time led to estimates of total forest dry deposition, including HNO_3, ranging from 5.7 to 19.1 kg N ha^{-1} $year^{-1}$ (Taylor et al., 1988a). However, Takemoto et al. (2001) pointed out that in parts of the mid-elevation forests of southern California, dry deposition rates may reach more than 40 kg N ha^{-1} $year^{-1}$. As a result, some locations have seen the conversion from N-limited to N-saturated forests. The concern for California's forests is well stated by Takemoto et al.: "As potential modifiers of long-term forest health, O_3 is a stressor and N deposition is an enhancer of ponderosa/Jeffrey pine physiology and growth (Grulke and Balduman, 1999). The progression toward a deciduous growth habit, higher shoot:root biomass ratios, increasing depths of litter, tree densification, and elevated NO_3^- levels in soil and soil solution, all point to the replacement of pine species with nitrophilous, shade- and O_3-tolerant tree species, such as fir and cedar (Minnich, 1999; Minnich et al., 1995)."

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

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

AX9.3.6.4 Hydrogen Fluoride and Other Gaseous Pollutants

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

the impact of O_3 on other species or even that the effect would ultimately have led to an effect on mature plants.

AX9.3.6.5 Acidic Deposition

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

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

With herbaceous species, Ashenden et al. (1995) noted significant antagonistic interactions of O_3 and acid mist in white clover, in which the adverse effect of low pH was countered by O_3. In contrast, Ashenden et al. (1996) found that, although pH 2.5 mist caused a significant stimulation of the growth of ryegrass attributed to a fertilizer effect, and O_3 caused reduced growth, there was no interaction. Bentgrass (*Agrostis capillaris*) behaved similarly.

A study by Bosley et al. (1998) on the germination of spores of the moss, *Polytrichum commune*, and the ferns, *Athyrium felix-femina* and *Onoclea sensibilis*, revealed no effect of O_3 on moss spores, while SAR at pH <4.0 was completely inhibitory. With the ferns, germination was progressively reduced by both increased O_3 and acidity.

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

AX9.3.6.6 Heavy Metals

As there appears to have been no further research into the interactions of oxidants with heavy metal pollutants, our understanding is unchanged from at the time of the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). As noted therein, the limited data available from early studies indicates varying degrees of enhancement of any adverse effects of O_3 but precludes the development of any response relationships.

AX9.3.6.7 Mixtures of Ozone with Two or More Pollutants

In many airsheds, the mixtures that occur, both concurrently and over time, may involve three or more pollutants. Very little useful information exists on the effects of O_3 with multiple pollutants. As the 1996 criteria document and others have pointed out, most of the early studies on such combinations can be discounted, because of their use of (1) high and environmentally irrelevant exposure concentrations and (2) unrealistic, repetitive exposure profiles (Barnes and Wellburn, 1998; U.S. Environmental Protection Agency, 1996).

The large investment in experimental facilities required to study these complex interactions is a major deterrent. So, although the topic has been included in several reviews that have appeared in the last decade, there appear to have been only two studies that have provided new information on the effects of O_3 in combination with more than one other pollutant stress. Ashenden et al. (1996) studied the effects of O_3 and/or (SO_2 + NO_2) with four acidities of SAR applied to each gas treatment, on white clover and two pasture grasses (*Lolium perenne* and *Agrostis capillaris*). With each species, the antagonism reported for the O_3 × SAR interaction

(Section AX9.3.4.6.5) tended to be nullified by concurrent exposure to the other gases, while the combination of the three gaseous pollutants resulted in the most severe growth inhibition, regardless of the acidity of the SAR.

With such meager evidence, no clear conclusions can be drawn as to the ways in which the effects of multiple airborne stressors could influence or be influenced by O_3.

AX9.3.7 Interactions with Agricultural Chemicals

The review of interactions involving O_3, plants, and various agricultural chemicals presented in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) remains a valid assessment of our limited knowledge of these interrelationships. Our knowledge is largely based on the protection against O_3 afforded to a range of crop species by applications of various chemicals, particularly fungicides, such as benomyl (benlate; methyl-1-[butylcarbamoyl]-2-benzimidazolecarbamate) and several carbamates and triazoles. A recent report has added azoxystrobin (AZO) and epoxyconazole (EPO) to the list (Wu and Tiedemann, 2002). Foliar sprays of either AZO or EPO provided 50 to 60% protection against O_3 injury to barley (*Hordeum vulgare*) leaves. Both had similar modes of action involving stimulation of the levels of antioxidant enzymes such as SOD, ascorbate peroxidase, guaiacol peroxidase, and catalase.

In contrast, applications of herbicides have yielded variable results ranging from increased sensitivity to protection from O_3; the nature of the effect is usually species- or cultivar-dependent. Although of less wide application, some plant growth retardants have also been found to provide protection, but no insecticide appears to have been clearly shown to have similar properties.

Despite the attraction of the use of permitted chemicals to provide crop protection, the statement in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) is still valid: "It is premature to recommend their use specifically for protecting crops from the adverse effects of O_3, rather than for their primary purpose."

AX9.3.8 Factors Associated with Global Climate Change

During the last decade, interest in the effects of climatic change on vegetation has replaced concerns over the purported causes of forest decline and the effects of acidic deposition. Two

specific components of climate change have been singled out as the foci of most of the research activity:

- the effects of increasing mean global CO_2 concentrations in the lower atmosphere, and
- the effects of increasing levels of surface-level irradiation by UV-B (the result of stratospheric O_3 depletion).

In spite of the crucial role of temperature as a climatic determinant (Monteith and Elston, 1993), the effects of increasing mean global temperatures and their interactions with increasing CO_2 levels in particular have received less attention.

All of the biotic and chemical interactions with oxidants discussed in the preceding sections may be modified by these climatic changes. However, research activities have largely focused on the two-way $O_3 \times CO_2$ interaction. Little if any experimental evidence exists related to three-way interactions, such as $O_3 \times CO_2 \times$ disease or $O_3 \times CO_2 \times$ nutrient availability, although such interactions are difficult to predict from the component two-way interactions.

Numerous reviews have appeared since the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) dealing with the issues involved. General reviews include publications of IPCC (1996, 2001); and UNEP (1993, 1999); the volume by Wellburn (1994); the volumes edited by Alscher and Wellburn (1994), De Kok and Stulen (1998), Singh (2000), and Yunus and Iqbal (1996); and papers by Idso and Idso (1994), Krupa and Groth (2000), Luo et al. (1999), Polle and Pell (1999), Poorter and Pérez-Soba (2001), Runeckles (2002), and Weubbles et al. (1999). Effects on agriculture and crop production, growth, and metabolism have been reviewed by Groth and Krupa (2000), Rötter and Van De Geijn (1999), and Schnug (1998); effects on forests have been reviewed by Bortier et al. (2000a); with focus on insect pests, Docherty et al. (1997), Karnosky et al. (2001a,c), McLaughlin and Percy (1999), and Saxe et al. (1998).

As background to the discussion of interactions with O_3, it should be noted that the increased levels of CO_2 experienced since the mid-18th century are such that, without abatement of the rates of increase, increased levels of from 540 to 970 ppm have been projected by the year 2100 (IPCC, 2001). Such increases in the concentration of CO_2, the principal GHG released into the atmosphere, will inevitably lead to increased global mean temperatures, evidence for which is already available from oceanic, icepack, and other records. The latest estimates of the global warming are for an increase in the range of 1.4 to 5.8 °C over this century, in contrast to the

0.6 °C rise experienced since 1900 (IPCC, 2001). However, considerable uncertainty is associated with such projections of future increases in global temperature.

The use of elevated CO_2 concentrations has been common practice for many years in the production of many greenhouse crops. Much of our early knowledge of the effects of higher than ambient CO_2 levels on plant growth derives from this application, coupled with research of plant physiologists on how CO_2 concentrations affect the process of photosynthesis. Information available about the effects of increased CO_2 levels on photosynthesis and stomatal function, in particular, has provided the underlying bases for numerous process models that simulate plant growth under stress and in changed climates.

Although simple O_3 × temperature interactions were discussed in Section AX9.3.4.2, the close linkage between global CO_2 levels and global mean temperatures in the context of climate change requires that an assessment of the interactive effects with O_3 should focus, as much as possible, on interactions involving all three factors.

AX9.3.8.1 Ozone-Carbon Dioxide-Temperature Interactions

Idso and Idso (1994) reviewed several hundred reports published between 1982 and 1994 on the effects of increased CO_2 on plant growth and net photosynthesis. Their survey covered a wide range of temperate and tropical, herbaceous and perennial species, including coniferous trees. They concluded that, for responses to a 300-ppm increase in CO_2, somewhat less than a doubling of present-day levels, but somewhat greater than the 540 ppm lower limit suggested by the IPCC (IPCC, 2001), averaged across all species:

- light intensity had a negligible effect on net photosynthesis other than at limiting low intensities under which the CO_2-driven enhancement was increased;
- increased temperature tended to increase the CO_2-driven enhancement of dry matter accumulation (growth) and net photosynthesis;
- drought conditions tended to increase the CO_2-driven enhancements of both growth and net photosynthesis, but increased salinity had little effect;
- mineral nutrient deficiency (especially of nitrogen) tended to increase the CO_2-driven enhancement of growth; and
- in the presence of air pollutants (especially SO_2 and NO_x), the CO_2-driven enhancement of net photosynthesis tended to be increased.

It should be noted that the statement that CO_2-enhanced growth increased with temperature referred to total dry matter accumulation by the whole plant and not to the yield of grain, fruit, or seed. Unfortunately, despite the existence of several reports at the time, the summary of interactions with air pollutants contained only a single reference to O_3, i.e., Pfirrmann and Barnes (1993), who reported surprisingly that a doubling of CO_2 levels led to a 27% increase in dry weight of radish but that the combination with O_3 led to a 77% increase.

The more recent reviews by Rudorff et al. (2000) and Olszyk et al. (2000) have addressed $CO_2 \times O_3$ interactions in detail, with the latter focusing on the implications for ecosystems. They concluded that:

- the effects of both gases on stomatal closure were predominantly additive, with little evidence of interaction;
- increased photosynthesis resulting from elevated CO_2 may be canceled by exposures to high O_3 levels;
- foliar O_3 injury is reduced by elevated CO_2; and
- interactions between CO_2 and O_3 can affect storage carbohydrates, leaf free-radical metabolism, and carbon allocation to shoots and roots.

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

Much of the recently published information on the effects of increased CO_2 and O_3 levels is summarized in Table AX9-12. Note that the table only lists the *directions* of O_3-induced effects and any modifications of these effects resulting from elevated CO_2, not their magnitudes. These directions are usually, but not necessarily, the same as the corollary effects of O_3 on CO_2-induced responses.

The bulk of the available evidence clearly shows that, under the various experimental conditions used (which almost exclusively employed abrupt or "step" increases in CO_2 concentration, as discussed below), increased CO_2 levels may protect plants from the adverse effects of O_3 on growth. This protection may be afforded in part by CO_2 acting together with O_3 in inducing stomatal closure, thereby reducing O_3 uptake, and in part by CO_2 reducing the negative effects of O_3 on Rubisco and its activity in CO_2-fixation. Although both CO_2-induced and O_3-induced decreases in stomatal conductance have been observed primarily in short-term

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

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
Biochemical/Metabolic					
Ascorbate peroxidase	∨	▼	Wheat (*T. aestivum*)	CSTR, P	Rao et al. (1995)
	∨	○	Sugar maple (*A. saccharum*)	CEC, P	Niewiadomska et al. (1999)
	∨	○	Trembling aspen (*P. tremuloides*)	FACE, G	Wustman et al. (2001)
Catalase	∨	○	Wheat (*T. aestivum*)	CEC, P	McKee et al. (1997b)
	∧	○		CEC, P	Niewiadomska et al. (1999)
	∨	○	Soybean (*G. max*)	OTC, P	Booker et al. (1997)
Chlorophyll	∨	▼	Wheat (*T. aestivum*)	OTC, G	Donnelly et al. (2000); Ommen et al. (1999)
	∨	▼	Potato (*S. tuberosum*)	OTC, G	Donnelly et al. (2001a)
	∨	▼	*Soybean (G. max)*		Heagle et al. (1998a); Reid and Fiscus (1998); Reid et al. (1998)
Glutathione reductase	∨	▼	Wheat (*T. aestivum*)	CSTR, P	Rao et al. (1995)
	○	○	Sugar maple (*A. saccharum*)	CEC, P	Niewiadomska et al. (1999)
	○	○	Aspen (*P. tremuloides*)	FACE, G	Wustman et al. (2001)
Glycolate oxidase	∨	▼	Soybean (*G. max*)	OTC, P	Booker et al. (1997)
Hydroxypyruvate reductase	∨	○	Soybean (*G. max*)	OTC, P	Booker et al. (1997)
Rubisco	∨	▼	Soybean (*G. max*)	OTC, P	Reid et al. (1998)
	∨	▼	Wheat (*T. aestivum*)	OTC, G	McKee et al. (2000)
	[∧]	▼	Trembling aspen (*P. tremuloides*)	FACE, G	Noormets et al. (2001b)
	∨	▼	Sugar maple (*A. saccharum*)	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

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
Biochemical/Metabolic (cont'd)					
Rubisco activity	⋁	▼	Soybean (*G. max*)	OTC, P	Reid et al. (1998)
	⋁	▼	Wheat (*T. aestivum*)	CEC, P	McKee et al. (1995)
	⋁	[▼]	Wheat (*T. aestivum*)	OTC, G	McKee et al. (2000)
	⋁	▼	European beech (*F. sylvatica*)	CEC, P	Lütz et al. (2000)
Superoxide dismutase	○	○	Wheat (*T. aestivum*)	CEC, P	McKee et al. (1997b)
	○	○	Sugar maple (*A. saccharum*)	CEC, P	Niewiadomska et al. (1999)
Physiological					
Stomatal conductance	⋁	▲	Radish (*R. sativus*)	CEC, P	Barnes and Pfirrmann (1992)
	⋁	▼	Soybean (*G. max*)	OTC, G	Mulchi et al. (1992)
	⋁	▲	Soybean (*G. max*)	OTC, P,G	Booker et al. (2005); Fiscus et al. (1997)
	⋁	▼	Bean (*P. vulgaris*)	OTC, P	Heagle et al. (2002)
	⋁	[▲]	White clover (*T. repens*) (O_3-sensitive)	CSTR, P	Heagle et al. (1993)
	⋀ - ○	▼ - ○	White clover (*T. repens*) (O_3-tolerant)	CSTR, P	Heagle et al. (1993)
	⋁	▲	Tomato (*L. esculentum*)	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
Physiological (cont'd)					
Stomatal conductance (cont'd)	⋁	▲	Potato (*S, tuberosum*)	OTC, G	Finnan et al. (2002)
	⋁	▲	Wheat (*T. aestivum*)	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)
	⋁	○▯	*Agropyron smithii*	CEC, P	Volin et al. (1998)
	⋁	○▯	*Koeleria cristata*	CEC, P	Volin et al. (1998)
	○▯	○▯	*Bouteloua curtipendula*	CEC, P	Volin et al. (1998)
	○▯	○▯	*Schizachyrium scoparium*	CEC, P	Volin et al. (1998)
	○▯	○▯	Black cherry (*P. serotina*)	CSTR, P	Loats and Rebbeck (1999)
	○▯	○▯	Green ash (*F. pennsylvanica*)	CSTR, P	Loats and Rebbeck (1999)
	[⋀]	▼	Yellow poplar (*L. tulipifera*)	CSTR, P	Loats and Rebbeck (1999)
	⋁	▲	Trembling aspen (*P. tremuloides*)	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
Physiological (cont'd)					
Stomatal conductance (cont'd)	○ - ⋁	▼		FACE, G	Noormets et al. (2001b)
	○	○	Red oak (*Q. rubra*)	CEC, P	Volin et al. (1998)
	[⋀]	▼	Durmast oak (*Q. petraea*)	CEC, P	Broadmeadow et al. (1999)
Photosynthesis	⋁	▼	Radish (*R. sativus*)	CEC, P	Barnes and Pfirrmann (1992)
	⋁	▼	Soybean (*G. max*)	OTC, P	Booker et al. (1997)
	⋁	▼	Soybean (*G. max*)	OTC, G,P	Mulchi et al. (1992)
	⋁	▼	Bean (*P. vulgaris*)	OTC, P	Heagle et al. (2002)
	⋁	○	Cotton (*G. hirsutum*)	OTC, P	Heagle et al. (1999b)
	⋁	○	Tomato (*L. esculentum*)	CEC, P	Hao et al. (2000)
	○	○	Potato (*S. tuberosum*)	OTC, G	Donnelly et al. (2001a)
	○	[▼]		OTC, G	Lawson et al. (2001b)
	[⋁]	▼	Wheat (*T. aestivum*)	CEC, P	Barnes et al. (1995)
	⋁	▼	Wheat (*T. aestivum*)	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

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
Physiological (cont'd)					
Photosynthesis (cont.)	○	○	*Agropyron smithii*	CEC, P	Volin et al. (1998)
	⋁	▼	*Koeleria cristata*	CEC, P	Volin et al. (1998)
	○ - ⋁	○	*Bouteloua curtipendula*	CEC, P	Volin et al. (1998)
	[⋁]	▼	*Schizachyrium scoparium*	CEC, P	Volin et al. (1998)
	⋁	▼	Ponderosa pine (*P. ponderosa*)	CEC, G	Olszyk et al. (2001)
	⋁	▼	Scots pine (*P. sylvestris*)	OTC, G	Kellomäki and Wang (1997a,b)
	○	○	Black cherry (*P. serotina*)	CSTR, P	Loats and Rebbeck (1999)
	[⋁]	▼	Green ash (*F. pennsylvanica*)	CSTR, P	Loats and Rebbeck (1999)
	○	○	Yellow poplar (*L. tulipifera*)	CSTR, P	Loats and Rebbeck (1999)
	⋁	▼	Trembling aspen (*P. tremuloides*)	CEC, P	Volin et al. (1998)
	⋁	▼	European beech (*F. sylvatica*)	CEC, P	Grams et al. (1999)
	○	○	Red oak (*Q. rubra*)	CEC, P	Volin et al. (1998)
	⋁	▼	Sugar maple (*A. saccharum*)	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

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
Physiological (cont'd)					
Photorespiration	∧	▼	Soybean (*G. max*)	OTC, P	Booker et al. (1997)
	∨	▲	Wheat (*T. aestivum*)	CEC, P	McKee et al. (1997b)
Growth, Yield					
Total biomass	∨	▼	Parsley (*P. sativum*)	CEC, P	Cardoso-Vilhena et al. (1998)
	∨	▼	Bean (*P. vulgaris*)	CEC, P	Cardoso-Vilhena et al. (1998)
	∨	▼		OTC, P	Heagle et al. (2002)
	∨	▼	Soybean (*G. max*)	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 (*M. sativa*)	CEC, P	Johnson et al. (1996a)
	∨	▼	White clover (*T. repens*) (O_3-sensitive)	CSTR, P	Heagle et al. (1993)
	○	○	White clover (*T. repens*) (O_3-tolerant)	CSTR, P	Heagle et al. (1993)
	∨	▼	Tomato (*L. esculentum*)	CEC, P CSTR, P	Hao et al. (2000) Reinert and Ho (1995)
	○	○	Potato (*S. tuberosum*)	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
Growth, Yield (cont'd)					
Total biomass (cont'd)	⋁	○		OTC, G	Lawson et al. (2001a)
	⋁	▼	Mustard (*S. alba*)	CEC, P	Cardoso-Vilhena et al. (1998)
	⋁	▼	Plantain (*P. major*)	CEC, P	Cardoso-Vilhena et al. (1998)
	⋁	▼	Cotton (*G. hirsutum*)	OTC, P	Booker (2000) Heagle et al. (1999b)
	⋁	▼	Wheat (*T. aestivum*)	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. (2000b) Rao et al. (1995) Rudorff et al. (1996a)
	[⋁]	▼	Wheat (*T. aestivum*)	OTC, G	Bender et al. (1999) Mulholland et al. (1997a)
	⋁	○	Wheat (*T. aestivum*)	CEC, P	Cardoso-Vilhena et al. (1998) Tiedemann and Firsching (2000)
	⋁	[▼]	Wheat (*T. aestivum*)	OTC, G	Ewart and Pleijel (1999)
	[⋁]	▼	Timothy (*P. pratense*)	CEC, P	Johnson et al. (1996a)
	⋁	▼	*Agropyron smithii*	CEC, P	Volin et al. (1998)
	⋁	▼	*Koeleria cristata*	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
Growth, Yield (cont'd)					
Total Biomass (cont'd)	∨	▼	Corn (*Z. mays*)	OTC, G	Rudorff et al. (1996a)
	∨	○	*Bouteloua curtipendula*	CEC, P	Volin et al. (1998)
	○	○	*Schizachyrium scoparium*	CEC, P	Volin et al. (1998)
	∨	▼	Ponderosa pine (*P. ponderosa*)	CEC, G	Olszyk et al. (2001)
	∨	▲	Birch (*B. pendula*)	CEC, P	Kytöviita et al. (1999)
	[∨]	○	Black cherry (*P. serotina*)	CSTR, P	Loats and Rebbeck (1999)
	∧	▼	Green ash (*F. pennsylvanica*)	CSTR, P	Loats and Rebbeck (1999)
	○	○	European ash (*F. excelsior*)	OTC, G	Broadmeadow and Jackson (2000)
	[∧]	▲	Yellow poplar (*L. tulipifera*)	CSTR, P	Loats and Rebbeck (1999)
	∨	▼	Sugar maple (*A. saccharum*)	CEC, P	Gaucher et al. (2003)
	∨	▼	Trembling aspen (*P. tremuloides*) (O_3-tolerant clone}	CEC, P OTC, P OTC, G	Volin et al. (1998) Dickson et al. (1998) Dickson et al. (2001)
	∨	○	Trembling aspen (*P. tremuloides*) (O_3-sensitive clone)	OTC, G	Dickson et al. (2001)
	○	○	Red oak (*Q. rubra*)	CEC, P	Volin et al. (1998)
	∨	▼	Durmast oak (*Q. petraea*)	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
Growth, Yield (cont'd)					
Total Biomass (cont'd)	○	○	Aleppo pine (*P. halepensis*)	CEC, P	Kytöviita et al. (1999)
	○	○	Scots pine (*P. sylvestris*)	OTC, G	Broadmeadow and Jackson (2000)
Seed/grain/fruit/tuber yield	⋁	▼	Soybean (*G. max*)	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 (*P. vulgaris*)	OTC, P	Heagle et al. (2002)
	⋁	▼	Tomato (*L. esculentum*)	CSTR, P	Reinert and Ho (1995)
	⋁	▼	Potato (*S, tuberosum*)	OTC, G	Finnan et al. (2002)
	○	○		OTC, G	Persson et al. (2003)
	⋁	▼	Wheat (*T. aestivum*)	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 (*T. aestivum*) (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

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
Growth, Yield (cont'd)					
Relative growth rate	⋁	▼	Wheat (*T. aestivum*)	CEC, P	Barnes et al. (1995)
	⋁	▼		CEC, P	Cardoso-Vilhena and Barnes (2001)
	⋁	▼	*Agropyron smithii*	CEC, P	Volin et al. (1998)
	⋁	▼	*Koeleria cristata*	CEC, P	Volin et al. (1998)
	○	○	*Bouteloua curtipendula*	CEC, P	Volin et al. (1998)
	○	○	*Schizachyrium scoparium*	CEC, P	Volin et al. (1998)
	○	○	European ash (*F. excelsior*)	OTC, G	Broadmeadow and Jackson (2000)
	⋁	▼	Trembling aspen (*P. tremuloides*)	CEC, P	Volin et al. (1998)
	[⋁]	▼	Red oak (*Q. rubra*)	CEC, P	Volin et al. (1998)
	⋁	▼	Durmast oak (*Q. petraea*)	OTC, G	Broadmeadow and Jackson (2000)
	○	○	Scots pine (*P. sylvestris*)	OTC, G	Broadmeadow and Jackson (2000)
Specific leaf area-SLA	⋁	○	Radish (*R. sativus*)	CEC, P	Barnes and Pfirrmann (1992)
	⋁	▼	Soybean (*G. max*)	OTC, P	Reid et al. (1998)
	⋁	▼	Cotton (*G. hirsutum*)	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

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
Growth, Yield (cont'd)					
Specific leaf area-SLA (cont'd)	∨	▲	White clover (*T. repens*) (O_3-sensitive)	CSTR, P	Heagle et al. (1993)
	∧	▲	White clover (*T. repens*) (O_3-tolerant)	CSTR, P	Heagle et al. (1993)
	∧	▲	*Agropyron smithii*	CEC, P	Volin et al. (1998)
	∧	▲	*Koeleria cristata*	CEC, P	Volin et al. (1998)
	○	○	*Bouteloua curtipendula*	CEC, P	Volin et al. (1998)
	∧	○	*Schizachyrium scoparium*	CEC, P	Volin et al. (1998)
	∧	▲	Trembling aspen (*P. tremuloides*)	CEC, P	Volin et al. (1998)
	∧	▲	Red oak (*Q. rubra*)	CEC, P	Volin et al. (1998)
Root/shoot ratio	∨	▼	Radish (*R. sativus*)	CEC, P	Barnes and Pfirrmann (1992)
	∨	▲	Alfalfa (*M. sativa*)	CEC, P	Johnson et al. (1996a)
	∨	▼	White clover (*T. repens*) (O_3-sensitive)	CSTR, P	Heagle et al. (1993)
	○	○	White clover (*T. repens*) (O_3-tolerant)	CSTR, P	Heagle et al. (1993)
	∧	▲	Wheat (*T. aestivum*)	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
Growth, Yield (cont'd)					
Root/shoot ratio (cont'd)	∨	▼	Timothy (*P. pratense*)	CEC, P	Johnson et al. (1996a)
	∨	○[▼]	Soybean (*G. max*)	OTC, G,P	Booker et al. (2005)
	○[▼]	○[▼]	Black cherry (*P. serotina*)	CSTR, P	Loats and Rebbeck (1999)
	○[▼]	○[▼]	Green ash (*F. pennsylvanica*)	CSTR, P	Loats and Rebbeck (1999)
	○[▼]	○[▼]	Yellow poplar (*L. tulipifera*)	CSTR, P	Loats and Rebbeck (1999)
	○[▼]	○[▼]	Aspen (*P. tremuloides*)	OTC, G	Dickson et al. (2001)
Foliar injury	∧	▼	Potato (*S. tuberosum*)	OTC, G	Donnelly et al. (2001b) Persson et al. (2003)
	∧	▼	Bean (*P. vulgaris*)	OTC, P	Heagle et al. (2002)
	∧	▼	Soybean (*G. max*)	OTC, P	Heagle et al. (1998a)
	∧	▼	Cotton (*G. hirsutum*)	OTC, P	Heagle et al. (1999b)
	∧	▼	Wheat (*T. aestivum*)	CEC, P OTC, G	Barnes et al. (1995) Mulholland et al. (1997a)
	∧	▼	Trembling aspen (*P. tremuloides*)	FACE, G	Karnosky et al. (1999) Wustman et al. (2001)
	∧	▼	European beech (*F. sylvatica*)	CEC, P	Grams et al. (1999)

O_3 m ≤ 0.15 ppm.

[b] CO_2-modifications of O_3-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 CO_2 enrichment facilities; OTC: open-top chambers. G: plants rooted in the ground; P: plants grown in pots. All species are C_3 except corn, *Bouteloua* and *Schizachyrium*.

studies, recent data clearly show a long-term and sustained reduction in stomatal conductance under elevated CO_2 for a number of species (Ainsworth and Long, 2005; Ellsworth et al., 2004; Gunderson et al., 2002). Instances of increased stomatal conductance have also been observed in response to O_3 exposure, suggesting partial stomatal dysfunction after extended periods of exposure (Maier-Maercker, 1998).

At the mechanistic level, Rubisco plays a key role in CO_2-assimilation, and while both O_3 and elevated CO_2 per se can lead to reduced activity, CO_2 can also reverse the O_3-induced inhibition of Rubisco activity and photosynthesis (Table AX9-12). However, in their review of the possible mechanisms involved, Polle and Pell (1999) cautioned that Rubisco should not be regarded "as a unique target for the interaction of the two gases." But it is clear from the bulk of the evidence in Table AX9-12 that elevated CO_2 levels can ameliorate the inhibition of growth caused by O_3 in many species, although the precise balance among the mechanisms involved may well vary from species to species. Three important caveats must be raised with regard to the findings presented in Table AX9-12:

- the applicability of results from experiments with an abrupt (step) increase in CO_2 level to understanding the consequences of the gradual increase in CO_2 predicted for the troposphere over the next hundred years;
- the validity of the findings in several long-term studies (particularly with tree species) conducted using potted plants, because of possible added stressors imposed on their root systems relative to trees growing in the field; and
- the relevance to understanding the effects of climate change of studies focused solely on CO_2 enrichment at current ambient conditions of temperature and precipitation patterns that provide no insights into possible interactive effects as these other climatic variables change concurrently with increasing CO_2 (Intergovernmental Panel on Climate Change [IPCC], 2001).

The first caveat concerns the distinctly different natures of the exposures to O_3 and CO_2 experienced by plants in the field. Changes in the ambient concentrations of these gases have very different dynamics. In the context of climate change, CO_2 levels increase relatively slowly and may change little over several seasons of growth. On the other hand, O_3 presents a fluctuating stressor with considerable hour-to-hour and day-to-day variability (Polle and Pell, 1999). Almost all of the evidence presented in Table AX9-12 comes from experimentation involving plants grown from the outset in, or subjected to, an abrupt or step increase to a higher

more or less double), steady CO_2 concentration. In contrast, the O_3 exposure concentrations usually varied from day to day. Luo and Reynolds (1999), Hui et al. (2002), and Luo (2001) noted the difficulties in predicting the likely effects of a gradual CO_2 increase from experiments involving a step increase or those using a range of CO_2 concentrations. Indeed, although using the much accelerated timescale of an 80-day growing season, Hui et al. (2002) clearly showed significant differences between the rates and magnitudes of various physiological and growth responses of plantain (*Plantago lanceolata*) to CO_2 between gradual and step increase treatments. The authors concluded that, even though there were major differences in most of the parameters studied between the gradual and step treatments, "the convergence of the measured parameters at the end of the experiment provides some encouragement for the applicability of step-type experiments in the field; however, the study suggests caution in interpreting early results from short-term studies."

In long-term studies, the matter of photosynthetic acclimation to elevated CO_2 levels has to be considered. Lawlor and Keys (1993) define *acclimation* in terms of long-term (days, weeks), irreversible physiological changes, in contrast to *regulation*, which relates to more rapid (minutes, hours), reversible changes. Each may be positive or negative, but many studies indicate that, while positive acclimation to elevated CO_2 levels initially led to enhanced photosynthesis and growth, negative acclimation ultimately ensued and reduced CO_2 assimilation and growth rates. However, the consensus from recent studies and reviews is that such negative acclimation is most likely to occur in situations in which plants are grown under some additional stress, induced, e.g., by limitations to growth posed by lack of resources such as water or nutrients. The meta-analysis by Curtis (1996) revealed that slow or little negative acclimation was noted in studies on unstressed tree species with unhindered opportunities for root growth and development, a view originally suggested by Arp and Drake (1991) and largely supported in the review by Eamus (1996). A nonwoody perennial, the rhizomatous wetland sedge, *Scirpus olneyi,* grown in its natural environment with no edaphic limitations showed no negative acclimation after 4 years; in fact, photosynthetic capacity increased by 31% (Arp and Drake, 1991). No negative acclimation of well-watered, field-grown Ponderosa pine trees was observed by Tissue et al. (1999) after 6 years of growth at 2×-ambient CO_2 levels. Gifford and Morison (1993) have summarized the situation thus: "Where the aerial or root environment for a plant is restricted (as with inter-plant competition, for example), positive feedback is limited and

adjustments to the changed resource input balance under high CO_2 can include 'down-regulation' of leaf photosynthesis rate as an integral part of a positive growth response."

The influence of other environmental stressors is borne out by several long-term tree studies. After 3 years in 565 ppm CO_2 in the Duke Forest FACE facility in North Carolina, maturing loblolly pine trees showed only a marginal CO_2-induced carbon gain if grown on a nutritionally moderate site, but zero gain if grown on a nutritionally poor site (Oren et al., 2001). This is in sharp contrast to the substantially increased initial growth rates in elevated CO_2 reported by DeLucia et al. (1999), but it is supported by the observations of Tognetti et al. (2000) on five Mediterranean tree species growing for many years adjacent to geothermal springs releasing CO_2 sufficient to provide ambient levels averaging 700 ppm. No significant differences in radial growth of the oaks (*Quercus cerris*, *Q. ilex*, and *Q. pubescens*), strawberry tree (*Arbutus unedo*), and flowering ash (*Fraxinus ornus*) could be detected between trees at the naturally enriched site and those at a nearby site exposed to normal ambient CO_2 (~350 ppm). The authors concluded that limited availability of water and nutrients may have counteracted any positive effects of CO_2 on growth at the enriched site or that the trees had acclimated to the higher CO_2 levels.

Because the ameliorative effects of CO_2 on responses to O_3 (Table AX9-14) were reported mostly in short-term studies involving an abrupt increase in CO_2 level, it is appropriate to ask whether this amelioration is likely to persist to a time when the ambient CO_2 concentration is relatively stable at such levels. Regardless of any negative acclimation due to resource limitations that may occur in the interim, steadily rising CO_2 levels may well lead to natural selection and genetic change. Nevertheless, it seems reasonable to expect that the amelioration of O_3 impact at elevated CO_2 levels will be maintained in many situations, but the negative acclimation that will probably occur in situations where other resources become limiting will reduce the degree of protection.

Another caveat regarding the validity of some of the observations in Table AX9-12 is related to the matter of stress-induced negative acclimation to elevated CO_2 and concerns related to using potted plants. Although much of the recent information on CO_2 effects has come from experiments with plants rooted in the ground, more than half of the studies listed in Table AX9-12 used potted plants, whether in controlled environment and greenhouse chambers or in OTCs. The degree to which pot-based studies resulted in similar patterns of response to

soil-grown plants appears to depend on the treatment conditions and plant growth conditions used in the study. The use of potted plants was a confounding factor in the studies of Taylor et al. (2001) of the differences in leaf growth of poplar (*Populus*) hybrids between plants exposed to elevated CO_2 in controlled environment chambers (potted plants), OTCs, or a FACE facility. Loats and Rebbeck (1999) suggested that their lack of CO_2 response in three broadleaf species may have resulted from their use of pot-grown plants. In contrast, Heagle et al. (1999a) found that the relative enhancement of soybean photosynthesis, growth, and yield by CO_2 enrichment was similar in pots and in the ground. These findings were supported by Booker et al. (2005). The recent meta-analysis of data on the effects of elevated CO_2 on soybean physiology and growth by Ainsworth et al. (2002) revealed a threefold smaller stimulation of seed yield in pot-grown than in field-grown plants, even when large (>9 L) pots were used. However, Ainsworth et al. (2002) included a wide range of treatment conditions (e.g., CO_2 treatments ranging from 500 to1200 ppm) and plant growth conditions in the meta-analysis, so caution is needed when generalizing conclusions about the applicability of pot-based studies.

Although the majority of the cases cited in Table AX9-12 indicate that O_3 and CO_2 act additively or synergistically in causing stomatal closure, there are numerous exceptions. Any reduction in stomatal aperture has consequences other than merely restricting O_3 uptake and the exchange of other gases. In particular, stomatal closure initially reduces the rate of transpiration, although increased leaf temperature and VPD associated with stomatal closure can offset decreases in transpiration. In instances where transpiration is reduced, water-use efficiency may increase, however decreased transpirational flux may lead to decreased mineral uptake, which could adversely impact growth over extended periods.

Hence, the final caveat regarding Table AX9-12 concerns the interactions of O_3 and CO_2 with other climatic variables, especially mean temperature. In light of the key role played by temperature in regulating physiological processes and modifying plant response to increased CO_2 levels (Long, 1991; Morison and Lawlor, 1999) and the knowledge that relatively modest increases in temperature may lead to dramatic consequences in terms of plant development (Lawlor, 1998), it is unfortunate that much of the large investment in time and resources spent on recent studies of the effects of climate change on vegetation have gone into investigations limited to increasing our knowledge of the effects of higher levels of CO_2 *at current ambient temperatures*.

Some attention is now being paid to investigating the concurrent effects of CO_2 increases and warming (recently reviewed by Rowland-Bamford [2000] and Morison and Lawlor [1999]), but the observed interactive effects on plant growth are inconsistent. For example, a FACE study with ryegrass (*Lolium perenne*) showed that increased temperatures (provided by infrared heaters) reduced the dry matter gain resulting from increased CO_2 levels (Nijs et al., 1996). The field studies by Shaw et al. (2002) on a California annual grassland dominated by the grasses *Avena barbata* and *Bromus hordeaceus* and the forbs *Geranium dissectum* and *Erodium botrys* involved free-air increased CO_2 as well as increased temperature, precipitation, and N supply. Not only did increased temperature reduce CO_2-stimulated net primary productivity (NPP), but increased CO_2 itself, combined with other factors, was found to be able to reduce NPP.

There have been several investigations of effects on wheat. Batts et al. (1997) used plastic tunnels to create temperature gradients and maintain elevated CO_2 levels over field-grown wheat and found that, in each of 4 years of study, a temperature rise of ~1.5 °C consistently canceled the growth and yield increases caused by a doubling of the CO_2 level above ambient. Similar findings were reported by Van Oijen et al. (1999) and Van Oijen and Ewart (1999) in OTC field studies. Half of the chambers were cooled 1.6 to 2.4 °C below the uncooled chambers, to cancel out the normal temperature increase over ambient, due to the so-called "chamber effect" (usually a 1 to 3 °C increase above ambient temperature (Heagle et al., 1988). Although temperature had no effect on CO_2-enhanced assimilation rates, the CO_2-enhanced growth and grain yields observed in the cooled chambers were effectively canceled out in the warmer chambers. The authors attributed this effect to accelerated phenology, a shorter period for grain filling, and a lower leaf area index (LAI; total leaf area per unit ground area) in the warmer chambers. Wheeler et al. (1996) observed that the benefit to wheat of doubling the CO_2 level was offset by a mean seasonal increase of only 1 to 1.8 °C. With the continuing use of OTCs for field research, Runeckles (2002), has suggested that the temperature rise due to the chamber effect in OTCs should be exploited (and measured) as a means of exploring temperature × CO_2 as well as temperature × CO_2 × O_3 interactions.

An indirect affirmation of the importance of temperature as a component of climate change on wheat yield was provided by Van Oijen and Ewart (1999) using two simulation models, AFRCWHEAT2-O_3 and LINTULCC (Ewart et al., 1999). They analyzed data from the ESPACE-wheat program, which involved 25 OTC experiments in 1994, 1995, and 1996 at nine

European locations (Jäger et al., 1999). Both models were able to predict control-treatment grain yields closely (5.5 ± 1.2 and 5.8 ± 1.2 $t \cdot ha^{-1}$, respectively, versus the observed 5.9 ± 1.9 $t \cdot ha^{-1}$), and both indicated a predominantly negative effect of temperature on the yield response to increased CO_2 (a 3 °C rise reduced the gain in yield from 30 to 14%). However, neither model had an $R^2 > 0.3$, indicating that the models included other sources of variability among the sites than the climatic factors. The multiple linear regression developed by Bender et al. (1999) based on the same datasets also included temperature as a highly significant covariant. Both studies are discussed more fully below.

Other studies, however, have found positive temperature-related growth effects, as suggested by the early Idso and Idso (1994) analysis. In an OTC study using the perennial grass *Festuca pratensis* in which a temperature increase of 3 °C above ambient was combined with CO_2-enrichment to 700 ppm, both CO_2 and temperature caused increases in total above-ground biomass (Hakala and Mela, 1996). Studies with potato (Cao et al., 1994) and soybean (Ziska and Bunce, 1997) using potted plants in controlled environment chambers also showed temperature-enhanced increases in growth in enriched CO_2 atmospheres. Read and Morgan (1996) compared the effects of enriched CO_2 and temperature on two grasses: cool-season *Pascopyrum smithii* and warm-season *Bouteloua gracilis*. In the latter (a C_4 species), 750 ppm CO_2 resulted in increased dry matter production at daytime temperatures as high as 35 °C, but in *P. smithii* (a C_3 species), CO_2-stimulated growth was greatest at 20 °C. However, the stimulation was progressively attenuated by increased temperature, so that at 35 °C, growth in 750 ppm was only one third of that in 350 ppm CO_2 at 20 °C.

Although the picture we have of temperature × CO_2 interactions is inconsistent, Rowland-Bamford (2000) has provided persuasive evidence that the nature of the response to temperature in the grain yield of crops with as different temperature optima as rice and wheat will depend upon whether the change is above or below the temperature optimum.

But what if we add O_3 as another variable? Unfortunately, there have been very few studies of the three-way interaction. With the information available on CO_2 × O_3 interactions (Table AX9-12) and the limited information on temperature × O_3 interactions (discussed in Section AX9.3.4.2) simulation modeling can attempt to provide estimates of O_3 × CO_2 × temperature effects, but experimental observation is still required to validate the models. The questions that need to be answered are: if increased temperature can offset the gains in

productivity afforded by increased CO_2 in important species such as wheat, and increased CO_2 can offset the reductions in productivity caused by O_3, will increased temperature modify this protective effect? And if so, in what manner?

To date, the only information available appears to consist of the reports by Van Oijen and Ewart (1999) and Bender et al. (1999) referred to above. In the former's simulation studies, the overall yield depression of wheat caused by O_3 was found to be 7 ± 4% for both AFRCWHEAT2-O_3 and LINTULCC models versus an observed 9 ± 11%. The enhancements due to CO_2 were predicted to be 24 ± 9% and 42 ± 11%, respectively, which straddled the observed 30 ± 22% gain. Based on the 13 experiments that included all four treatments (±O_3, ±CO_2), an actual 10% yield loss due to O_3 at ambient CO_2 levels was reduced to a 4% loss by the elevated CO_2. The AFRCWHEAT2-O_3 model predicted 7 and 4% losses, and LINTULCC model predicted 8 and 5% losses due to the O_3 and O_3 + CO_2 treatments. The actual and simulated yield increases in response to CO_2 increased further with increasing temperature, but although temperature had no discernible effect on the observed depression of yield caused by O_3 alone, both models suggested that the yield reduction was diminished both by higher temperatures and higher CO_2 levels.

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

$$Y = 1004.6^{***} + 0.588^{***}[CO_2] - 1.908^{**}[O_3] - 31.230^{***}[T] + 7.309[I] - 1448.423^{***}[H_2O], \quad (9\text{-}5)$$

where Y = grain yield, $g \cdot m^{-2}$; $[CO_2]$ = ppm CO_2; $[O_3]$ = ppb O_3, 12-h mean; $[T]$ = °C; $[I]$ = light intensity, $MJ \cdot m^{-2}$/day; and $[H_2O]$ is a dummy variable: well watered = 1; limited water supply = 2. (***, $p < 0.001$; **, $p < 0.01$; the coefficient for I was not significant). With R^2 = 0.3983, adjusted for 258 degrees of freedom, a large part of the variability was still unaccounted for by the five variables. However, this analysis suggests that CO_2, O_3, temperature, and water-status are important codeterminants of wheat yield, but assumes no interactions. Substitution in the model at summer light intensities and with well-watered plants indicates that, at 20° C, a doubling of CO_2 levels to 700 ppm alone would lead to a 29.5% increase in yield, while 50 ppb O_3 alone would decrease yield by 10.9%. With both gases at those levels, the yield

would only increase 20%, but with a concurrent temperature rise of 2 °C, it would shrink to a 9.6% increase.

Both studies, therefore, indicate an amelioration of the effects of O_3 by CO_2, the magnitude of which would be reduced at warmer temperatures. However, they relate to a single crop whose response to CO_2 is temperature-sensitive. Information about other species in which the effects of CO_2 and temperature are additive are limited. However, Wolf and Van Oijen (2003) recently described a model (LPOTCO) simulating the effects of changes in climatic variables, CO_2 and O_3 on tuber yield potential of irrigated potato (*S. tuberosum* cv. Bintje) over locations within the European Union ranging from Finland to Italy. They noted that although increased CO_2, O_3, and light intensity were predominant controlling factors, increased temperature also influenced potential yields substantially, with increases in northern latitudes (attributed to a longer growing season) but decreases in southern latitudes (attributed to decreased assimilate production).

A clear understanding of the complex interactions of increased CO_2 and temperature with O_3 must await further experimentation or simulations. However, it seems likely that any CO_2-induced amelioration of the adverse effects of O_3 on aspects of growth other than seed or grain yield may be lessened or increased by increased temperature, depending upon the temperature optima for the species, along the lines suggested by Rowland-Bamford (2000).

Other crop simulation models which incorporate O_3 and some of the various environmental factors, including elements of climate change, have been reviewed by Kickert et al. (1999) and Rötter and Van De Geijn (1999). However, to date, the applications tend to have focused on interactions of O_3 with factors such as soil moisture or nutrient availability.

With forest trees, the situation has the added complexity of a perennial growth form and the inevitability, over time, of subjection to additional environmental stresses such as nutrient-limitation. Here, too, although numerous models of tree growth have been described, there appear to have been few applications to interactions of O_3 and factors of climate change. Constable et al. (1996) used TREGRO to model the growth of Ponderosa pine exposed to three O_3 levels (0.5×, 1.0×, and 2× ambient), two levels of CO_2 (ambient and ambient + 200 ppm CO_2), and two temperature regimes (ambient and ambient + 4 °C). Plant growth was predicted to be decreased 1, 19, and 39% by the three levels of O_3, respectively. Increased CO_2 reduced the loss at the highest O_3 level to 7%; however, the combination of elevated CO_2 with the higher temperature more than overcame the adverse effects of O_3, leading to a 4% increase,

largely attributed to increased fine root mass. The authors suggested that, in relation to the baseline conditions used in the simulations (Corvallis, OR), higher concentrations of CO_2 and O_3 and a warmer climate will have little impact on total-tree growth, but they noted the importance of undertaking multiple stress studies in order to be able to make accurate forecasts of the impact of such changes on forest trees.

More recently, Constable and Friend (2000) compared the capabilities of six published process-based models (CARBON, ECOPHYS, PGSM, TRE-BGC, TREGRO, and W91) for simulating tree response to elevated CO_2, O_3, and temperature. They concluded that, although these models were capable integrators of the effects of various environmental factors on individual processes such as photosynthesis, they were less reliable when extrapolating to growth.

Although most of the research emphasis has been on simple $CO_2 \times O_3$ interactions, a few isolated studies of interactions have involved O_3, CO_2, and biotic environmental factors. Heagle et al. (1994a) observed that both O_3 and CO_2 tended to be additive in encouraging the growth of spider mite (*Tetranychus urticae*) populations on clover. Infection of wheat with leaf rust (*Puccinia recondita*) sensitized the plants to O_3 injury, but its severity was significantly reduced in elevated CO_2 (Tiedemann and Firsching, 2000). The effects of O_3 and CO_2 on mycorrhizal symbioses was studied by Kytöviita et al. (1999) who found that CO_2 did not ameliorate the adverse effects of O_3 on the root growth of Aleppo pine and European white birch. In another study with Aleppo pine, Kytöviita et al. (2001) noted that both O_3 and elevated CO_2 reduced mycorrhiza-induced N-uptake by the roots. In Scots pine, Kasurinen et al. (1999) observed transient effects of elevated CO_2 and O_3 on root symbiosis, but none of the effects persisted over the 3 years of the study.

The soil water $\times O_3 \times CO_2$ interaction was experimentally investigated by Broadmeadow and Jackson (2000) in Durmast oak, European ash, and Scots pine. No interactions were noted with ash and pine; but with oak, elevated CO_2 ameliorated and irrigation exacerbated the effects of O_3, although the resultant effects were essentially additive.

Booker (2000) noted that soil nitrogen levels interacted only slightly with O_3 and CO_2 in determining the composition of cotton leaves and roots. Carbon dioxide reversed the inhibition of leaf growth caused by O_3, but increased N-fertility tended to reduce this reversal.

Because of the small number of studies of possibly significant interactions of three or more environmental factors, it is impossible to draw any sweeping conclusions as to how O_3, in the context of global climate change, may affect relationships among plants and insects, diseases, and symbionts or among plants and nutrients or other air pollutants. The only interaction that has some degree of general support is the amelioration of adverse O_3 effects by elevated CO_2.

AX9.3.8.2 Ozone-UV-B Interactions

As noted in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), depletion of stratospheric O_3 by halofluorocarbons has resulted in increased intensities of UV-B radiation (280 to 320 nm wavelengths) at the Earth's surface. The situation is discussed more fully in Chapter 10.

While stratospheric O_3 depletion may result in increased surface UV-B irradiation, absorption of UV-B is a property of the O_3 molecule regardless of its location; surface UV-B flux is, therefore, also reduced by O_3 in the troposphere. Although only about 10% of the total atmospheric O_3 column occurs in the troposphere (Fishman et al., 1990), it contributes a disproportionately greater absorption effect than stratospheric O_3, because the UV radiation penetrating the troposphere becomes increasingly diffuse as it reaches the surface, with a consequent increase in mean path length (Brühl and Crutzen, 1989). Any benefits to vegetation from reduced ambient O_3 stress must, therefore, also be viewed in the context of possible adverse effects due to increased UV-B irradiation. There are, thus, two distinct types of possible interactions between surface level O_3 and UV-B radiation:

- direct interactions involving simultaneous, sequential, or mixed exposures to O_3 and UV-B stresses; and
- effects on responses to UV-B itself resulting from changes in radiation intensity caused by changes in surface-level O_3 concentrations.

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

The most recent reviews specifically addressing the *combined* effects of tropospheric O_3 and UV-B on plants are by Runeckles and Krupa (1994) and Krupa and Jäger (1996), although the topic has also been included in several more general reviews of O_3 effects and factors of

climate change, such as those by Unsworth and Hogsett (1996), Krupa et al. (1998), Posthumus (1998), and Krupa and Groth (2000).

However, little new information has become available since Runeckles and Krupa (1994) noted that the scanty knowledge of the effects of UV-B and O_3 combinations available at that time was derived solely from studies of soybean. Miller et al. (1994) observed no interaction and no effect of UV-B on yield, in contrast to a previous report by Teramura et al. (1990) using the same cultivar, Essex. More recently, in a study of the saltmarsh grass *Elymus athericus* subjected to reciprocal exposures to O_3 and UV-B, Van De Staaij et al. (1997) observed no interactive effects and no adverse effects of UV-B following 14-day exposures, even though an earlier report showed that longer exposures to UV-B (65 days) could cause a 35% loss of biomass (Van De Staaij et al., 1993). However, in a study in which ambient, high-altitude UV-B levels were compared with near-zero levels, at ambient or 2×-ambient levels of O_3, interactions involving the levels of antioxidants in Norway spruce and Scots pine were reported by Baumbusch et al. (1998). Schnitzler et al. (1999) subsequently reported that O_3-induced injury and adverse effects on photosynthesis were more pronounced with near-zero UV-B levels, indicating an amelioration of the O_3-response. A later study with Scots pine (Zinser et al., 2000) revealed $O_3 \times$ UV-B interactions at the gene expression and biochemical levels. In contrast, Ormrod et al. (1995) reported that UV-B predisposed *Arabidopsis thalliana* to injurious growth effects from O_3 exposure.

At various organizational levels, Runeckles and Krupa (1994) identified several similarities between plant response to O_3 and UV-B, and at the level of gene expression, there have recently been several reports of both similarities and distinctions. Willekens et al. (1994) reported similar effects of O_3, UV-B, and SO_2 on the expression of antioxidant genes in *Nicotiana plumbaginifolia*. For parsley (*Petroselinum crispum*), Eckey-Kaltenbach et al. (1994a) found that O_3 was a cross-inducer for both the UV-B-induced enhanced biosynthesis of flavonoids and the pathogen-induced furanocoumarin phytoalexins, in keeping with the previously observed O_3-induction of fungal and viral defense reactions. In this regard, Yalpani et al. (1994) provided evidence that O_3 and UV-B acted similarly in increasing disease-resistance via a salicylate-mediated enhancement of defense proteins in tobacco. However, subsequent work with tobacco led Thalmair et al. (1996) to conclude that exposure to UV-B did not lead to the accumulation of pathogenesis-related proteins. In Scots pine, although O_3 is known to induce

stilbene synthase and cinnamyl alcohol dehydrogenase, UV-B was found to enhance the former but suppress the latter, revealing an interaction at the level of gene expression (Zinser et al., 2000).

In summary, the present base of information about possible interactions between increased UV-B radiation and O_3 is insufficient to draw any firm conclusions in terms of gross effects, but there is some evidence of similarities in the effects of O_3 and UV-B individually and of the mechanisms involved at the molecular level.

AX9.3.8.3 Interactions of Ozone with Multiple Climate Change Factors

Despite the need for experimental investigations of three-way or more complex interactions among O_3, CO_2, UV-B, temperature, and other climate change factors, few studies have been reported, even without O_3 as a factor. In an isolated report, using tomato seedlings, Hao et al. (2000) employed preexposure to UV-B ($\pm CO_2$ enrichment) followed by exposure to O_3 ($\pm CO_2$ enrichment). They observed that CO_2 enrichment more than overcame the inhibition of photosynthesis caused by O_3, but pretreatment with UV-B reduced the resultant increase.

In view of the unexpected observations made in their grassland study of the combined effects of CO_2, temperature, precipitation, and N-supply, Shaw et al. (2002) affirmed that "Ecosystem responses to realistic combinations of global changes are not necessarily simple combinations of the individual factors." The addition of O_3 to the list of variables results in further complexity.

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

AX9.3.9 Summary - Environmental Factors

Although O_3 and other photochemical oxidants are phytotoxic, their actions on vegetation may be modified by a host of biotic and abiotic factors in the environment; conversely, they may modify plant response to these other factors. The extensive review of these biological, physical, and chemical factors conducted for the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) concluded with a statement that our understanding was too fragmented to permit drawing

many general conclusions. With today's increased awareness of the need for more complete information on interactions, it is unfortunate that, in the interval since the 1996 O_3 AQCD, rigorous, systematic investigations of interactions have been rare, and most of the new information is as fragmented as before. This is inevitable, partly in view of the vast scope of the possible interactions between O_3 and other environmental variables and partly due to the overall lack of funding for such research in these areas.

In the area of biotic interactions, new evidence with regard to insect pests and diseases has done little to remove the uncertainties noted in the 1996 criteria document. Most of the large number of such interactions that may affect crops, forest trees, and other natural vegetation have yet to be studied. The trend suggested previously that O_3 increases the likelihood and success of insect attack has received some support from recent studies, but only with respect to chewing insects. With the economically important group of sucking insects such as the aphids, no clear trends have been revealed by the latest studies. Hence, although it seems likely that some insect problems could increase as a result of increased O_3 levels, we are still far from being able to predict the nature of any particular O_3 plant insect interaction, its likelihood, or its severity.

The situation is a little clearer with respect to interactions involving facultative necrotrophic plant pathogens with O_3, generally leading to increased disease. With obligate biotrophic fungal, bacterial, and nematode diseases, there are twice as many reports indicating O_3-induced inhibitions than enhancements. The frequent reports that infection by obligate biotrophs reduces the severity of O_3-induced foliar injury should not be interpreted as "protection", because of the negative effects on the host plant of the disease per se. With obligate biotrophs, the nature of any interaction with O_3 is probably dictated by the unique, highly specific biochemical relationships between pathogen and host plant. At this time, therefore, although some diseases may become more widespread or severe as a result of exposure to O_3, it is still not possible to predict which diseases are likely to present the greatest risks to crops and forests.

Several studies have indicated that the functioning of tree root symbioses with mycorrhizae may be adversely affected by O_3, but there is also evidence that the presence of mycorrhizae may overcome root diseases stimulated by O_3 and that O_3 may encourage the spread of mycorrhizae to the roots of uninfected trees. The latest studies, therefore, present no clearer picture of the likely nature of simple interactions of O_3 and root symbionts, but in view of the importance of

mycorrhizae as below-ground components of ecosystems, they are discussed more fully in Section AX9.5.

The few recent studies of the impact of O_3 on intraspecific plant competition have again confirmed that grasses frequently show greater resilience than other types of plants. In grass-legume pastures, the leguminous species suffer greater growth inhibition. And the suppression of Ponderosa pine seedling growth by blue wild-rye grass was markedly increased by O_3. However, we are far from being able to predict the outcome of the impact of O_3 on specific competitive situations, such as successional plant communities or crop-weed interactions.

Light, a component of the plant's physical environment, is an essential "resource" whose energy content drives photosynthesis and CO_2 assimilation. It has been suggested that increased light intensity may increase the sensitivity to O_3 of light-tolerant species while decreasing that of shade-tolerant species, but this appears to be an oversimplification with many exceptions. Temperature affects the rates of all physiological processes based on enzyme-catalysis and diffusion, and each process and overall growth (the integral of all processes) has a distinct optimal temperature range. Although some recent field studies have indicated that O_3 impact significantly increases with increased ambient temperature, other studies have revealed little effect of temperature. But temperature is unquestionably an important variable affecting plant response to O_3 in the presence of the elevated CO_2 levels contributing to global climate change (see below). In contrast, evidence continues to accumulate to indicate that exposure to O_3 sensitizes plants to low temperature stress by reducing below-ground carbohydrate reserves, possibly leading to responses in perennial species ranging from rapid demise to impaired growth in subsequent seasons.

Although the relative humidity of the ambient air has generally been found to increase the adverse effects of O_3 by increasing stomatal conductance and thereby increasing O_3 flux, abundant evidence indicates that the ready availability of soil moisture results in greater sensitivity to O_3. The partial "protection" against the adverse effects of O_3 afforded by drought has been observed in field experiments and modeled in computer simulations. There is also compelling evidence that O_3 can predispose plants to drought stress. Hence, the response will depend to some extent upon the sequence in which the stresses occur, but, even though the nature of the response is largely species-specific, successful applications of model simulations will lead to larger-scale predictions of the consequences of $O_3 \times$ drought interactions. However,

it must be recognized that regardless of the interaction, the net result on growth in the short-term is negative, although in the case of tree species, other responses such as increased water use efficiency could be a benefit to long-term survival.

Mineral nutrients in the soil, other gaseous air pollutants, and agricultural chemicals constitute chemical factors in the environment. The evidence regarding interactions with specific nutrients is still contradictory. Some experimental evidence indicates that low general fertility increases sensitivity to O_3, while simulation modeling of trees suggests that nutrient deficiency and O_3 act less than additively; however there are too many example of contrary trends to permit any sweeping conclusions. Somewhat analogously with temperature, it appears that any shift away from the nutritional optimum may lead to greater sensitivity, but the shift would have to be substantial before a significant effect on response to O_3 was observed.

Interactions of O_3 with other air pollutants have received relatively little recent attention. The situation with SO_2 remains inconsistent, but seems unlikely to pose any additional risk to those related to the individual pollutants. With NO and NO_2, the situation is complicated by their nutritional value as N sources. In leguminous species, it appears that NO_2 may reduce the impact of O_3 on growth, with the reverse in other species, but the nature of the exposure pattern, i.e., sequential or concurrent, also determines the outcome. Much more investigation is needed before we will be able to predict the outcomes of different O_3-NO-NO_2 scenarios. The latest research into O_3 × acid rain interactions has confirmed that, at realistic acidities, significant interactions are unlikely. A continuing lack of information precludes offering any generalizations about interactive effects of O_3 with NH_3, HF, or heavy metals. More evidence has been reported that the application of fungicides affords some protective effects against O_3.

Over the last decade, considerable emphasis has been placed on research into O_3 interactions with the components of global climate change: increased atmospheric CO_2, increased mean global temperatures, and increased surface-level UV-B radiation. However, many of these studies have tended to regard increased CO_2 levels and increased mean temperatures as unrelated phenomena. Experiments into the effects of doubled CO_2 levels at today's mean ambient temperatures may not reveal the impact of climate change on responses to O_3. For example, the limited experimental evidence and evidence obtained by computer simulation suggest that in a 600+ ppm world, although the enriched CO_2 would more than offset the impact of O_3 on responses as varied as wheat yield or the growth of young Ponderosa pine

trees, the concurrent increase in temperature would reduce, but probably not eliminate, the net gain. A similar decrease in the net gain resulting from the complete reversal by CO_2 of the inhibition of photosynthesis caused by O_3 has been reported for increased UV-B irradiation. Clearly, additional research is needed in this important area.

In conclusion, although the increased use of computer simulations may be important in suggesting outcomes of the many complex interactions of O_3 and various combinations of environmental factors, the results obtained will only be as reliable as the input data used for their parameterization. The data needed for good simulations can only come from organized, systematic study. For predicting the future, ignorance is as good as dependence on poor simulations.

AX9.4 EFFECTS-BASED AIR QUALITY EXPOSURE INDICES

AX9.4.1 Introduction

Indices are metrics that relate measured plant damage (i.e., reduced growth) to monitored ambient O_3 concentration over time. An index is needed to provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. Such indices may also provide a basis for developing a biologically-relevant air quality standard for protecting ecological resources. Effects on plant growth and/or yield has been a major focus of the characterization of O_3 impacts on plants for purposes of the air quality standard setting process (U.S. Environmental Protection Agency, 1986, 1996b). The quantifying function over some time frame has frequently been referred to as "dose-response" and "exposure-response." The distinction is in how the pollutant concentration is measured: "dose" is the measured pollutant concentration absorbed by the leaf over some time period, whereas "exposure" is the ambient air concentration measured near the plant over some time period.

A measure of plant O_3 uptake from the ambient air (either rate of uptake or cumulative seasonal uptake) is the ideal measure, because without O_3 or its reactive product(s) reaching the target tissue there is no effect (Tingey and Taylor, 1982). Uptake is controlled in part by stomata (see Section AX9.2 for a detailed discussion). An uptake measure should integrate all those environmental factors that influence stomatal conductance, e.g., temperature, humidity, soil water status. A direct measure of the internal leaf concentration of O_3, however, is technically

difficult and thus uptake values are generally obtained with simulation models that require species- and site-specific variables. Because of this, a surrogate for uptake was sought early on using statistical summaries of monitored ambient pollutant concentration over some integral of time (Lee et al., 1988; Lefohn and Benedict, 1982; O'Gara, 1922; U.S. Environmental Protection Agency, 1986, 1992, 1996b).

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

AX9.4.2 Summary of Conclusions from the Previous Criteria Document

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

Studies prior to 1996 indicated that the components of exposure, including concentrations, temporal dynamics (e.g., time of day of peak events), frequency of occurrence, duration, and respite time, were integral to developing indices of exposure related to growth response. Evidence from the few direct experimental studies of varying exposure components indicate the importance of peak concentrations, temporal pattern of occurrence, respite time and the

importance of cumulating the concentrations over the exposure period (Hogsett et al., 1985a; Musselman et al., 1983, 1986, 1994; U.S. Environmental Protection Agency, 1996b).

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

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

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

An exposure index that incorporated either the daily or seasonal temporal patterns of higher concentration occurrence with the temporal pattern of individual species' stomatal conductance was not reported in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996b). Based on available data, it was unresolved how to proceed with weighting time of day or temporal patterns of species conductance.

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

The composite exposure-response functions for crops and tree seedlings were derived from single and multiyear exposure studies that used modified or simulated ambient exposure profiles. These profiles were typified by episodic occurrence of a large number of higher O_3 concentrations. This type of pattern is not atypical but is not found in all rural agricultural and some forested areas in the United States. Selecting a concentration value from these crop and seedling response models may result in an over- or underestimation of growth effects if applied to regions of the country where a different type of temporal pattern of occurrence is prevalent (U.S. Environmental Protection Agency, 1996b). A multicomponent index was suggested that combined the concentration-weighted, cumulative index with the number of occurrences of hourly averaged concentrations $\geq$0.10 ppm that might reduce the uncertainty associated with selecting the exposure value for protection based on NCLAN-type studies (Lefohn and Foley, 1992; Musselman et al., 1994; U.S. Environmental Protection Agency, 1996b). No direct experimental studies addressed this question prior to 1996, nor have any since.

Since 1996, additional research has focused on the time of day when the higher hourly average concentrations occur, the time of day of maximum plant uptake, the diurnal variability of plant defense mechanisms, and various suggestions as to including these factors in any one of the cumulative, concentration-weighted exposure indices. A much broader literature has focused since 1996 on relating O_3 flux to plant response and how to use this as an index relating ambient concentration to effects. These new developments are discussed in the sections that follow.

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

Mathematical approaches for summarizing ambient air quality information in biologically meaningful forms that can serve as surrogates for dose for O_3 vegetation effects assessment purposes have been explored for more than 80 years (O'Gara, 1922; U.S. Environmental Protection Agency, 1996b). Several indices have attempted to incorporate some of the biological, environmental, and exposure factors (directly or indirectly) that influence the magnitude of the biological response and contribute to observed variability (Hogsett et al., 1988). In the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996b), the exposure indices were arranged into five categories; (1) One Event, (2) Mean, (3) Cumulative, (4) Concentration Weighted, and (5) Multicomponent, and were discussed in detail (Lee et al., 1989). Figure AX9-16 illustrates how several of the indices weighted concentration and accumulate exposure.

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

Most of the early retrospective studies reporting regression approaches used data from the NCLAN program or data from Corvallis, Oregon or California (Lee et al., 1987; Lee et al., 1988; Lefohn et al., 1988; Musselman et al., 1988; U.S. Environmental Protection Agency, 1992; U.S. Environmental Protection Agency, 1986). These studies were previously reviewed by the EPA (U.S. Environmental Protection Agency, 1992, 1996b) and were in general agreement that the best fit of the data were cumulative concentration-weighted exposure indices. Lee et al. (1987) suggested that exposure indices that included all the 24-h data performed better than those that

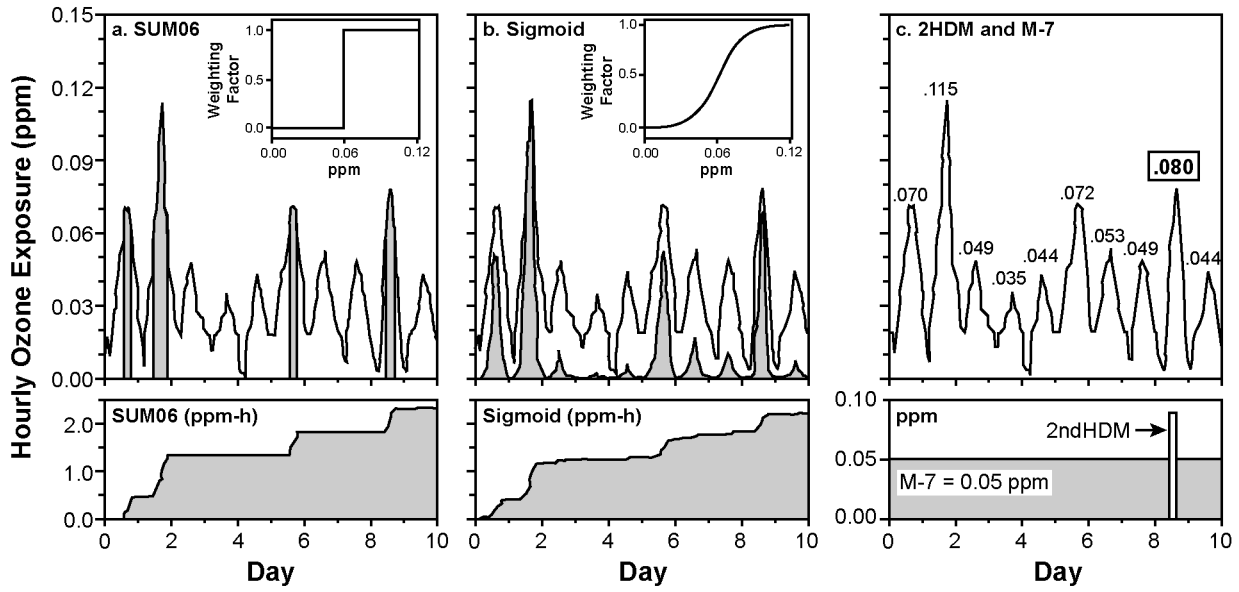


Figure AX9-16. Diagrammatic representation of several exposure indices, illustrating how they weight concentration and accumulate exposure. (a) SUM06: the upper graphic illustrates an episodic exposure profile; the shaded area under some of the peaks illustrates the concentrations greater than or equal 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).

used only 7 h of data; this was consistent with the conclusions of Heagle et al. (1987) that plants receiving exposures for an additional 5-h/day showed 10% greater yield loss than those exposed for 7-h/day. In an earlier analysis using the NCLAN data, Lee et al. (1988) found the "best" exposure index was a phenologically weighted cumulative index, with sigmoid weighting on concentration and a gamma weighting function as a surrogate for plant growth stage. This index

was the best statistical fit, but it depended upon a greater knowledge of species and site conditions making specification of weighting functions difficult for general use.

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

A statistical approach based on profile likelihoods was used to estimate parameters in generalized exposure indices similar to the SUM06 and AOT40 indices (Blankenship and Stefanski, 2001) using data from experiments conducted during 1993 at eight sites in the eastern United States in which O_3-sensitive and -tolerant white clover genotypes were grown using methods developed by Heagle et al. (Heagle et al., 1994b). The results showed that for the SUMX family of indices, where X is a cutoff value, hourly O_3 concentrations over ~71 ppb contribute the most to yield prediction. For the AOTX family of indices, the parameter was 54.4 ppb. These values are similar to those used in the SUM06 and AOT40 indices already in use. Furthermore, investigation of weighting for time of day confirmed the importance of the mid-afternoon hours for this data set, unlike the results found for wheat in Sweden (Danielsson et al., 2003; Pleijel et al., 2000a).

Other factors, including predisposition time (Hogsett et al., 1988; McCool et al., 1988) and crop development stage (Heagle et al., 1991; Tingey et al., 2002), contributed to variation in the biological response and suggested the need for weighting O_3 concentrations to account for predisposition time and phenology. However, the roles of predisposition and phenology in plant response vary considerably with species and environmental conditions, so specification of a weighting function for general use in characterizing plant exposure was not possible.

European scientists took a similar approach in developing indices describing growth and yield loss in crops and tree seedlings, using OTCs with modified ambient exposures, but many fewer species and study locations were employed in the European studies. There is evidence from some European studies that a lower (Pleijel et al., 1997) or higher (Finnan et al., 1996, 1997) cutoff value may provide a better statistical fit to the experimental data. Finnan et al. (1997) used seven exposure studies of spring wheat to confirm that cumulative exposure indices emphasizing higher O_3 concentrations were best related to plant response and that cumulative exposure indices using weighting functions, including cutoff concentrations, allometric and sigmoidal, provided a better fit and that the ordering of these indices differed with different

linear or Weibull dose-response models. Weighting those concentrations associated with sunshine hours in an attempt to incorporate a element of plant uptake did not improve the index performance (Finnan et al., 1997). A more recent study using data from several European studies of Norway spruce, analyzed the relationship between relative biomass accumulation and several cumulative, weighted indices, including the AOT40 and the SUM06 (Skärby et al., 2004). All the indices performed relatively well in regressing biomass and exposure index, with the AOT20 and AOT30 doing slightly better (r^2 = 0.46-0.47). In another comparative study of four independent data sets of potato yield and different cumulative uptake indices with different cutoff values, a similarly narrow range of r^2 was observed (0.3 -0.4) between the different cumulative uptake of O_3 indices (Pleijel et al., 2002).

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

The main conclusions from the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996b) regarding an index based on ambient exposure are still valid. No information has come forth in the interim to alter those conclusions significantly, and in fact, some recent studies have further substantiated them. These key conclusions can be restated as follows:

- O_3 effects in plants are cumulative;
- higher O_3 concentrations appear to be more important than lower concentrations in eliciting a response;
- plant sensitivity to O_3 varies with time of day and crop development stage;
- exposure indices that accumulate the O_3 hourly concentrations and preferentially weight the higher concentrations have better statistical fits to growth/yield response than do the mean and peak indices.

Following the 1996 criteria review process (U.S. Environmental Protection Agency, 1996a,b), the EPA proposed an alternative form of the secondary NAAQS for O_3 using a cumulative, concentration-weighted exposure index to protect vegetation from damage (Federal

Register, 1997). The EPA considered three specific concentration-weighted indices: the cutoff concentration weighted SUM06 and AOT60 and the sigmoid-weighted W126 exposure index (U.S. Environmental Protection Agency, 1996a). All three indices performed equally well in predicting the exposure-response relationships observed in the crop and tree seedlings studies conducted during the previous 20 years (Lee et al., 1989). In a workshop convened to consider the science supporting these indices (Heck and Cowling, 1997), the participants agreed that all the cumulative concentration- weighted indices considered were equally capable of predicting plant response.

The cutoff concentration-weighted index AOT40 was selected for use in Europe in developing exposure-response relationships based on OTC studies of a limited number of crops and trees (Grünhage and Jäger, 2003). The United Nations Economic Commission for Europe (UNECE, 1988) adopted the critical levels approach for assessment of O_3 risk to vegetation across Europe. As used by the UNECE, the critical levels are not air quality regulatory standards in the U.S. sense, but rather planning targets for reductions in pollutant emissions to protect ecological resources. Critical levels for O_3 are intended to prevent long-term deleterious effects on the most sensitive plant species under the most sensitive environmental conditions, but not to quantify O_3 effects. A critical level was defined as "the concentration of pollutant in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems, or materials may occur according to present knowledge" (UNECE, 1988). The nature of the "adverse effects" was not specified in the original definition, which provided for different levels for different types of harmful effect (e.g., visible injury or loss of crop yield). There are also different levels for crops, forests, and seminatural vegetation. The caveat, "according to present knowledge," is important because critical levels are not rigid; they are revised periodically as new scientific information becomes available. For example, the original critical level for O_3 specified concentrations for three averaging times, but further research and debate led to the current critical level being stated as the cumulative exposure (concentration × hours) over a cutoff concentration of 40 ppb (AOT40) (Fuhrer et al., 1997). The level of 3 ppm·h was selected, corresponding to a 5% yield loss in spring wheat as determined from 15 OTC studies. The critical level was defined for a 3-month period calculated for daylight hours. This value is currently used for all crops, because it is the best supported value and because the limited data from other crop species do not provide strong evidence that a more stringent value is required

(Fuhrer et al., 1997). "Level I" critical levels are currently used to map and identify areas in Europe in which the levels are exceeded, and that information is then used to plan optimized and effects-based emissions abatement strategies. In the 1990s, areas of exceedance were mapped, but analyses of many exposure studies led to the conclusion that the simple, exposure-based approach led to the overestimation of effects in some regions and underestimation in others (Fuhrer et al., 1997; Kärenlampi and Skärby, 1996). The "Level I" approach did not differentiate between plant species, and it did not include modifying site and such micrometeorological factors of O_3 uptake as VPD, water stress, temperature, and light and variation in canopy height.

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

AX9.4.4 Identifying Exposure Components That Relate to Vegetation Effects

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

AX9.4.4.1 Role of Concentration

A significant role of higher concentrations was established earlier, based on several experimental studies (U.S. Environmental Protection Agency, 1996b). Several studies since the last review (Nussbaum et al., 1995b; Oksanen and Holopainen, 2001; Yun and Laurence, 1999a) have added support for the important role that peak concentrations, as well as the pattern of occurrence, plays in plant response to O_3. Oksanen and Holopainen (2001) found that the peak concentrations and the shape of the O_3 exposure (i.e., duration of the event) were important determinants of foliar injury in European white birch saplings, but growth reductions were found to be more related to total cumulative exposure. Based on air quality data from 10 U.S. cities, three 4-week exposure treatments having the same SUM06 value were constructed by Yun and Laurence (1999a). They used the regimes to explore effects of treatments with variable versus uniform peak occurrence during the exposure period. The authors reported that the variable peak exposures were important in causing injury, and that the different exposure treatments, although having the same SUM06, resulted in very different patterns of foliar injury. Nussbaum et al. (1995b) also found peak concentrations and the pattern of occurrence to be critical in determining the measured response. The authors recommended that to describe the effect on total forage yield, peak concentrations >0.11 ppm must be emphasized by using an AOT with higher threshold concentrations.

A greater role for higher concentrations affecting plant growth might be inferred based on recent air quality analyses for the Southern California area (Lee et al., 2003; Tingey et al., 2004). In the late 1960s and 1970s, extremely high O_3 concentrations had impacted the San Bernardino NF. However, over the past 15 plus years, significant reductions in O_3 exposure have occurred in the San Bernardino National Forest (Davidson, 1993; Lee et al., 2003; Lefohn and Shadwick, 2000; Lloyd et al., 1989). An illustration of this improvement in air quality is shown by the 37-year history of O_3 air quality at a site in the San Bernardino Mountains (Figure AX9-17) (Lee et al., 2003). The O_3 exposure increased from 1963 to 1979 concurrent with increased population and vehicular miles, followed by a decline to the present mirroring decreases in precursor emissions. The pattern in exposure was evident in various exposure indices including the cumulative concentration weighted (SUM06), as well as maximum peak event (1-h peak), and the number of days having hourly averaged O_3 concentrations $\geq$95 ppb (i.e., the California O_3 standard). The number of days having hourly averaged O_3 concentrations $\geq$95 ppb

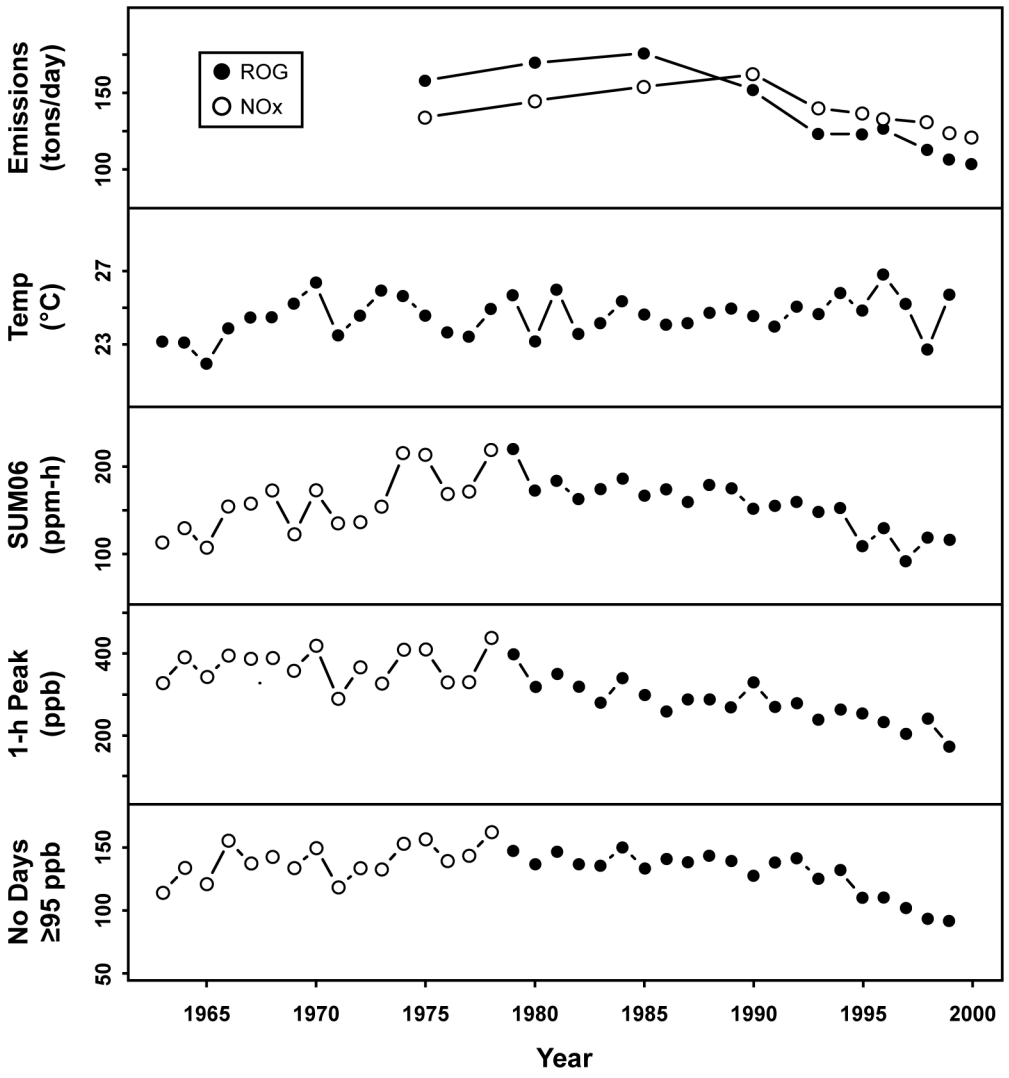


Figure AX9-17. Trends in May to September 12-h SUM06, peak 1-h O_3 concentration and number of daily exceedances of 95 ppb for Crestline in 1963 to 1999 in relation to trends in mean daily maximum temperature for Crestline and daily reactive organic gases (ROG) and oxides of nitrogen (NO_x) for San Bernardino 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).

declined significantly from 163 days in 1978 to 103 days in 1997. The changes in ambient O_3 air quality for the site were reflected in the changes in the frequency and magnitude of the peak hourly concentration and the duration of the exposure (Figure AX9-17). Considering the role of exposure patterns in determining response, the seasonal and diurnal patterns in hourly O_3 concentration did not vary appreciably from year to year over the 37-year period (Lee et al., 2003).

The inference for a role of higher concentrations comes both from results of ground measures of tree conditions on established plots and from results of model simulations. Across a broad area of the San Bernardino NF, the Forest Pest Management (FPM) method of injury assessment indicated an improvement in crown condition from 1974 to 1988; and the area of improvement in injury assessment is coincident with an improvement in O_3 air quality (Miller and Rechel, 1999). A more recent analysis of forest changes in the San Bernardino NF using an expanded network of monitoring sites has verified significant changes in growth, mortality rates, basal area, and species composition throughout the area since 1974 (Arbaugh et al., 2003). A model simulation of Ponderosa pine growth over the 40-year period in the San Bernardino NF showed a significant impact of O_3 exposure on tree growth and indicates improved growth with improving O_3 air quality. This area has also experienced elevated N deposition and based on a number of environmental indicators, it appears that this area is experiencing N saturation (Fenn et al., 1996). To account for this potential interaction, the model simulations were conducted under conditions of unlimited soil N, which helps account for these effects. The actual interactions are not known. The improvement in growth was assigned to improved O_3 air quality, but no distinction was made regarding the relative role of mid-range and higher hourly concentrations, only that improved growth tracked decreasing SUM06, maximum peak concentration, and number of days of hourly $O_3 \geq 95$ ppb (Tingey et al., 2004). A summary of air quality data from 1980 to 2000 for the San Bernardino NF area of the number of "mid-range" hourly concentrations indicated no dramatic changes over this 20-year period, ranging from about 1500 to 2000 hours per year (Figure AX9-18). There was a slow increase in the number of mid-range concentrations from 1980 to 1986, which corresponds to the period after implementation of the air quality standard. Another sharper increase was observed in the late 1990s. This pattern of occurrence of mid-range hourly concentrations suggests a lesser role for

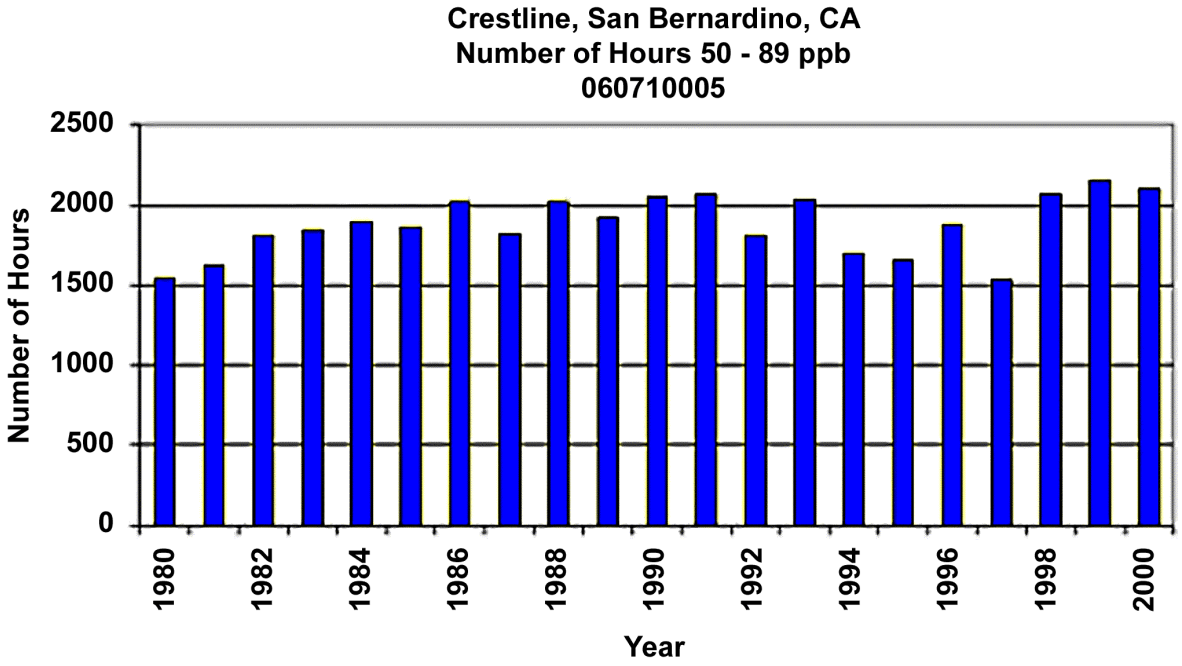


Figure AX9-18. The number of hourly average concentrations between 50 and 89 ppb for the period 1980 to 2000 for the Crestline, San Bernardino, CA monitoring site.

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

these concentration ranges compared to the higher values in either of the ground-level tree injury observations of the model simulation of growth over the 40-year period.

AX9.4.4.2 Role of Duration

Recent studies have called into question the period of time over which concentrations are accumulated and the form of the exposure index. Heagle and Stefanski (2000) reported that the form of the exposure index was important only for 24-h indices for which SUM00 (cumulated all hourly concentrations with no concentration weighting) provided the poorest fit. The authors reported that the SUM00, SUM06, W95 (Lefohn and Runeckles, 1987), W126, and AOT40 produced similarly good fits of the foliage biomass data for 6-, 5-, and 4-h midday accumulating periods. The study pooled data from San Bernardino (CA) and Riverside (CA) with data from Amherst (MA), Corvallis (OR), Kennedy Space Center (FL), Raleigh (NC), and Blacksburg (VA). Ozone exposures were much higher at the two California sites (indicated by high W126,

SUM06, W95, and AOT40 values) compared to the other locations. Because of the pooling of the data, the large number of high hourly average O_3 concentrations that occurred at the California sites may have resulted in the exposure indices being highly correlated with one another and made it difficult to identify one optimal index.

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

AX9.4.4.3 Patterns of Exposure

A significant factor in developing exposure indices is the temporal patterns of O_3 occurrence over a day, a month, and a year, as well as seasonally overlaying the daily and seasonal temporal patterns of those influential climatic and site factors. The coincidence of peak O_3 with maximal stomatal conductance and detoxification processes is key to affecting plant growth response (Musselman and Minnick, 2000).

Daily Patterns

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

A 12-h daylight period for cumulating exposure was proposed following the 1996 O_3 AQCD based primarily on the assumption that most species probably do not have significant conductance at night (U.S. Environmental Protection Agency, 1996a). An extensive review of the literature, however, reported that a large number of species had varying degrees of nocturnal stomatal conductance (Musselman and Minnick, 2000). The role of nighttime stomatal

conductance and O_3 exposure was demonstrated experimentally as well. Grulke et al. (2004) showed that the stomatal conductance at night for Ponderosa pine in the San Bernardino NF (CA) ranged from one tenth to one fourth that of maximum daytime gas exchange. In June, at the high-elevation site, 11% of the total daily O_3 uptake of pole-sized trees occurred at night. In late summer, however, O_3 uptake at night was negligible. Birch seedlings exposed to O_3 at night show greater reductions in growth than those exposed to O_3 in daylight (Matyssek et al., 1995). Field mustard (*Brassica rapa* L.) plants exposed to O_3 during the day or night showed little significant difference in the amounts of injury or reduced growth response to O_3 treatment, although the stomatal conductance was 70 to 80% lower at night (Winner et al., 1989). Tissue biomass of Ponderosa pine seedlings was significantly reduced when seedlings were exposed to either daytime or nighttime episodic profiles (Lee and Hogsett, 1999). However, the biomass reductions were much greater with daytime peak concentrations than with nighttime peak concentrations.

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

Stomatal conductances are species-dependent and linked to both diurnal and seasonal meteorological activity as well as to soil/site conditions (e.g., soil moisture). Daily patterns of leaf/needle conductance were often highest in midmorning, whereas higher ambient O_3 concentrations generally occurred in early to late afternoon when stomata were often partially

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

The generalized models of stomatal conductance may provide a means to link patterns of O_3 occurrence with climatic and site factors that affect O_3 uptake, provided conductance is modeled by regions of similar seasonal moisture and by similar canopy structure (Emberson et al., 2000a, 2000b) (Grünhage et al., 2000) (Massman, 2004).

Seasonal Patterns

Several of the recent studies measuring O_3 flux to pine canopies also reported on the importance of seasonal patterns in relating exposure to response (Bauer et al., 2000). These seasonal patterns can be early- versus late-season occurrence of higher O_3 concentrations, reflecting climate and precursor availability. The patterns also reflected seasonal drought and the role soil moisture played in stomatal conductance and O_3 uptake. Recently, studies looked directly at this linkage. Panek et al. (2002) compared direct O_3 flux measurements into a canopy

of Ponderosa pine with a number of exposure indices and demonstrated a lack of correlation, especially in the late-season drought period; the authors concluded that a consideration of climate, especially soil moisture, was essential. They suggested that a better metric for seasonally drought-stressed forests would be one that incorporates forest physiological activity, through mechanistic modeling, by weighting ambient O_3 concentrations by stomatal conductance, or by weighting O_3 concentrations by site moisture conditions. Panek (2004) demonstrated a decoupling of O_3 exposure and uptake seasonally as well, via seasonal drought influence. Maximum O_3 uptake occurred at the beginning of the season and in the winter, whereas the pines were nearly dormant during August to September.

Using TREGRO, a process-based tree growth model, Tingey et al. (2004) simulated long-term growth of Ponderosa pine over a 37-year period. The simulation showed a high degree of association between O_3 exposure and O_3-induced reductions in tree growth ($R^2 = 0.56$). The scatter about the line, however, indicated that other factors beside O_3 are required to describe the association between exposure and response. Incorporating annual temperature and precipitation increased the R^2 to 0.67. In keeping with the observations of Panek (2004) on the decoupling of peak O_3 occurrence and maximal conductance, the remaining unexplained variation is attributed to differences in timing of peak O_3 uptake and peak O_3 exposure over the years.

AX9.4.4.4 Frequency of Occurrence of Peak Concentrations

Several earlier studies demonstrated the greater effect of episodic occurrence of O_3 peaks compared to daily peak events (U.S. Environmental Protection Agency, 1996b). Since the 1996 O_3 AQCD, a few studies have corroborated the importance of this pattern in growth response (Köllner and Krause, 2003; Yun and Laurence, 1999a; Nussbaum et al., 1995b). Köllner and Krause (2003) reported that, under equal exposure conditions, the most pronounced effects on the yield of sugar beet (*Beta vulgaris* L.) and soybeans occurred with those regimes that emphasized the episodic occurrence of peak events. Similarly, Yun and Laurence (1999b) used exposure regimes constructed from 10 U.S. cities to demonstrate that variable peak occurrence versus uniform occurrence was important in causing injury in tree seedlings. Nussbaum et al. (1995b) compared the effects of different patterns of peak occurrences with similar AOT40 values and reported a stronger effect on total forage yield from the episodic treatment.

AX9.4.4.5 Canopy Structure

Another factor important in either O_3 exposure or uptake is how canopy structure affects O_3 concentration in and under forest canopies. There have been several investigations of O_3 concentrations under tree canopies (Enders, 1992; Fontan et al., 1992; Fredericksen et al., 1995; Joss and Graber, 1996; Kolb et al., 1997; Lorenzini and Nali, 1995; Neufeld et al., 1992; Samuelson and Kelly, 1997). In general, they indicated a reduction in O_3 of ~20 to 40% in the area below the canopy but above the shrub/herb layers. An essential component in the determination of the AOT40 as a critical level was the height at which the O_3 concentration was measured. The measurement heights are related to the O_3 concentration measured at the top of the canopy, i.e., upper surface boundary of the (quasi-) laminar layer (Grünhage and Jäger, 2003). This location is presumably more related to stomatal uptake. Weighting the O_3 concentration at this location takes into account stomatal opening and, if weighted with the Jarvis-Steward factors for radiation, temperature, and soil moisture, the "toxicologically" effective AOT40 is obtained (Grünhage and Jäger, 2003). A question exists however as to whether this "canopy" O_3 concentration is clearly connected to stomatal O_3 uptake. During site conditions that limit stomatal conductance (e.g., low soil moisture, high VPD) at the top of the canopy, high concentrations of O_3 can occur with minimal risk.

In a study that considered those factors important in O_3 uptake that are also spatially distributed as a result of canopy structure, Davison et al. (2003) reported that the variation in visible injury in coneflower (*Rudbeckia laciniata* var. *digitata*) populations in Great Smoky Mountains National Park was unlikely to be due to differences in O_3 flux and more likely due to variation in PAR. At a height of 50 cm above ground, PAR was reduced by almost 90%, whereas the O_3 varied from about 15 to 90% of ambient. Ozone injury was not solely related to O_3 flux. Although there have been studies of the effects of different light levels on O_3 response, there have been few at the very low levels that occur in canopies of tall herbaceous stands or in the ground layer of forests. Davison et al. (2003) reported that conductance was not related to diurnal changes in light. The O_3 levels were still about 90% of the O_3 concentration above the canopy when light was less than 5%. Light intensity dropped to 1.5% of open at 130 cm from the edge of the canopy, while O_3 dropped to only 42%. The study, although reporting on the adequacy of visible foliar injury as an indicator of O_3 effects, suggested that consideration of

other factors such as light were important in predicting response. How this might be included in developing exposure-response indices was not considered.

AX9.4.4.6 Site and Climate Factors

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

AX9.4.4.7 Plant Defense Mechanism - Detoxification

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

AX9.4.5 Ozone Uptake or Effective Dose as an Index

Another approach in developing an index that relates growth response to ambient O_3 is based on determining the O_3 concentration going from the atmosphere into the leaf, or flux. Interest has been increasing in recent years, particularly in Europe, in using mathematically tractable flux models for O_3 assessments at the regional and national scale (Emberson et al.,

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

AX9.4.5.1 Models of Stomatal Conductance

Only a limited number of studies have measured O_3 concentration or its reaction products within the leaf (e.g., Moldau and Bichele (2002); see Section AX9.4.3). Altimir et al. (2002) described an enclosure technique for measuring O_3 flux to foliage at the shoot level that allowed determination of partitioning and seasonality of the removal pathways on the foliage. The loss of O_3 to the wall material of the chamber was great and required a correction when the stomatal activity was low. Only a few instances of direct measures of O_3 flux to foliage in the field have been reported. Most measures of O_3 flux are from canopy measurements made with micrometeorological techniques, but a number of assumptions are necessary and there are

limitations due to landscapes (Grünhage et al., 2000; Wesely and Hicks, 2000). Comparison of simulated and measured O_3 flux densities show good agreement in the mean (Grünhage et al., 2000). Comparison, however, of continuous O_3 concentrations and fluxes measured over a 5-year period by the gradient method (Fowler et al., 1989) in a 30-year-old Norway spruce stand demonstrated a correlation over 5 years but were not correlated on a diurnal or seasonal basis. The correlation was based on two uncoupled processes inside and outside the stomata, i.e., the destruction of O_3 outside the stomata in the canopy was influenced by those same factors (temperature, light, humidity) that control the diurnal opening and closing of the stomata. A similar lack of correlation of measured concentration and estimated flux, daily and seasonally, into Norway spruce and cembran pine (*Pinus cembra*) at six sites was due mostly to the control of stomatal conductance by those same microenvironmental factors (temperature, humidity, irradiance) (Emberson et al., 2000b). Seasonal variation of flux was attributed to the temperature course. During the growing season, the leaf-air VPD was the environmental factor controlling stomatal conductance and O_3 flux into the needles.

Given the limitations of actual measures of flux and the lack of correlation between measured concentrations and flux, a key goal is to develop an uptake or flux-based response index using models that consider site, climatic, meteorological, and species-specific (e.g., detoxification reactions) factors. Models of O_3 conductance into plant tissue are available (Grünhage et al., 1997; Massman, 1993; Wesely, 1989). The European Monitoring and Evaluation Program (EMEP) developed an O_3 deposition model for application across Europe in conjunction with the EMEP photochemical model as a tool for the critical levels program (Emberson et al., 2000a). The model was developed to estimate vegetation type-specific O_3 deposition and stomatal flux, calculated according to a standard three-resistance formulation incorporating atmospheric, boundary layer, and stomatal resistances (Emberson et al., 2000a). The model used a multiplicative algorithm of the stomatal conductance of O_3 (Jarvis, 1976) and has been parameterized for 10 European tree species, seven crop species, and one type of seminatural vegetation. The model calculates conductance as a function of leaf phenology, temperature, photosynthetic flux density (PFD), VPD, and soil moisture deficit (SMD). The environmental variables are site-specific (or regionally-specific). The most important factors limiting O_3 with this model were VPD, SMD, and phenology (Emberson et al., 2000a). These

factors demonstrate the critical linkage of high VPD and stomatal closure, which typically co-occur with high O_3 concentrations.

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

The complexity of using flux as an index of O_3 exposure for growth response is shown in field studies that measured O_3 flux into Norway spruce and cembran pine (Emberson et al., 2000b). They demonstrated that stomatal conductance was the main limiting factor for O_3 uptake and showed the dependence of that measure on crown position, needle age, and altitude. Consideration of the role of climate illustrates the importance of a flux measure. Pleijel et al. (2000b) reported the improved relationship of yield in spring and winter wheat grown in OTCs in many areas across Europe when it was related to the cumulative stomatal O_3 uptake during the grain-filling period. Compared to the AOT40, the cumulative uptake index estimated larger yield losses in the relatively humid parts of western and northern Europe, while smaller yield loss was estimated for the dry summer climates in southern and central Europe.

Danielsson et al. (2003) compared the ability of two different stomatal models to relate grain yield in field-grown spring wheat to cumulated O_3 uptake and an exposure index of AOT40 and found that the cumulated O_3 uptake determined with either model performed better in relating exposure to yield than did the cumulative exposure index of AOT40.

Cumulative O_3 uptake (CU) was modeled for three deciduous and two coniferous species growing at different sites and elevations and compared with the AOT40 exposure measure at these sites (Matyssek et al., 2004). A general linearity was demonstrated between the two measures of O_3 exposure, and, at any given AOT40, there was a 25 ± 11% variation in CU. Although no correlation of growth alterations was observed with either the exposure or the uptake measure, the modeled cumulative uptake was able to describe the variation in tree size and site location, making for a better measure in risk assessment of O_3 (Matyssek et al., 2004).

Karlsson et al. (2004b) compared the biomass-response relationship in young trees at seven experimental sites across Europe using modeled cumulative O_3 uptake and AOT40. A weaker dose-response relationship was reported for the cumulative uptake metric compared to the AOT40 (Karlsson et al., 2004b).

Concern about the complexity of the stomatal models and the data needed to model O_3 uptake has led some researchers to offer modified accumulated exposure indices that consider the meteorological factors controlling uptake (Gerosa et al., 2004; Karlsson et al., 2004a). In a study of subterranean clover in Austria, Belgium, and southern Sweden, Karlsson et al. (2004a) reported on the performance of a modified accumulated exposure over the threshold (mAOT) which was based on solar radiation and VPD. This index improved the relationship for observed visible injury. But when modeled uptake of O_3 was derived from a simple stomatal conductance model considering solar radiation, VPD, and air temperature, this index gave an even greater improvement in the relationship to visible injury than did the ambient exposure index of AOT40 (Karlsson et al., 2004a). The added value of the mAOT was worthwhile, as was its lower degree of complexity and data requirements compared to simulating O_3 uptake with stomatal models. Based on a study of O_3 fluxes over a barley (*Hordeum vulgare* L.) field in Italy, a similar modified exposure index was reported and referred to as "effective exposure" (Gerosa et al., 2004). Their approach was similar in its consideration of physiological aspects in conjunction with monitored O_3 concentrations. It also addressed the shortcomings of the data needs for modeled O_3 uptake.

Models that partition O_3 uptake into stomatal and non-stomatal components are also now available and predict a significant non-stomatal component in calculating O_3 flux (Altimir et al., 2004; Bassin et al., 2004; Mikkelsen et al., 2004; Nikolov and Zeller, 2003; Nussbaum et al., 2003; Zeller and Nikolov, 2000). Altimir et al. (2004) compared the relative contributions of stomatal and non-stomatal sinks at the shoot level for Scots pine. Using the EMEP model with a revised parameterization for Scots pine, they demonstrated that a major removal of O_3 was due to the non-stomatal component; when a non-stomatal term was introduced dependent on ambient relative humidity, the non-stomatal contribution to the total conductance was about 50%. Zeller and Nikolov (2000) demonstrated a large non-stomatal O_3 uptake (41% of the total annual flux) in subalpine fir at a site in southern Wyoming using the biophysical model FORFLUX. In a 5-year study of measured O_3 flux to a Norway spruce canopy, Mikkelsen et al. (2004) showed

monthly patterns of non-stomatal and stomatal deposition as part of total deposition to the canopy. Their study demonstrated that daily means of O_3 concentration and fluxes averaged over 5 years correlated well, but the correlation was based on two different noncoupled processes outside and inside the stomata. The destruction of O_3 in the canopy was influenced by temperature, light, and humidity, and these same factors influence stomatal opening, e.g., midday and night closure. Consequently, the diurnal O_3 concentration and O_3 flux do not correlate at all during the growing season. The study estimated yearly stomatal uptake to be a minimum of 21% of total deposition (i.e., non-stomatal uptake was as high as 80% of total). The stomatal uptake was highest May to August (30 to 33%) and lowest November to February (4 to 9%).

AX9.4.5.2 Nonlinear Response and Developing Flux Indices

If O_3 flux were used as the only metric to predict vegetation injury or damage, the prediction might be overestimated, because of nonlinear relationships between O_3 and plant response (Amiro et al., 1984; Amiro and Gillespie, 1985; Bennett, 1979, 1996b, 1986; U.S. Environmental Protection Agency, 1978). The nonlinearity in the response surface suggests the existence of a biochemical threshold. Musselman and Massman (1999) suggested that those species having high amounts of detoxification potential might show less of a relationship between O_3 stomatal uptake and plant response. More recently, nonlinear relationships between O_3 flux and yield were shown for potato (Pleijel et al., 2002) and spring wheat (Danielsson et al., 2003). The relationship between O_3 flux and potato yield led to the use of an instantaneous flux threshold to overcome the nonlinear relationship (Pleijel et al., 2002). However, the authors did not report a substantial improvement in the mathematical fitting of the model when applying the threshold. Most of the flux was accumulated below 0.06 ppm. However, Danielsson et al. (2003) showed an improved relationship between O_3 uptake and yield of spring wheat using a threshold of 5 nmoles m^{-2} sec^{-1} (0.24 mg m^{-2} sec^{-1}). These results suggest not all O_3 entering the stomata contribute to a reduction in yield, which depends to some degree on the amount of internal detoxification occurring for each particular species (see Section AX9.2). The cellular detoxification reactions and repair processes which both detoxify oxidants as well as play central roles in the carbon economy of the plant are another level of resistance to O_3 reaching the target tissue (see Section AX9.2). The magnitude of the response is

determined by the amount of the pollutant reaching the target site and the ability of the plant to reestablish homeostatic equilibrium. Thus, one would expect to observe a decoupling of O_3 uptake with vegetation effects, which would manifest as a nonlinear relationship between O_3 flux and injury or damage.

Additional factors for inclusion in flux-based models to predict vegetation effects would be the defense and repair mechanisms. However, the fact that the defense and repair mechanisms vary diurnally as well as seasonally may make it extremely difficult to apply a mathematically determined threshold to instantaneous flux measurements to calculate cumulative flux. The threshold models do not allow for the temporal (i.e., daily and seasonal) variability of defense mechanisms. Specifically, the relationship between conductance, O_3 concentration, and defense/repair mechanisms needs to be included. Recently, Massman (2004) illustrated that the combination of stomatal conductance, O_3 concentration, and diurnal variation of defense mechanisms showed the daily maximum potential for plant injury (based on effective dose) coincided with the daily peak in O_3 mixing ratio. Massman et al. (2000) stressed that the product of the overlapping mathematical relationships of conductance, concentration, and defense mechanisms results in a much different picture of potential impact to vegetation than just the use of conductance and concentration in predicting vegetation effects.

AX9.4.5.3 Simulation Models

Another approach for determining O_3 uptake and relating growth response to ambient O_3 exposure may be the use of physiologically-based simulation models. Several of these have been used in various contexts, comparing O_3 response in a number of tree species with varying climate and site factors (e.g., soil moisture) (Hogsett et al., 1997; Laurence et al., 2001; Ollinger et al., 1997, 1998; Weinstein et al., 2001, 2002). These process-based models provide for an integration of species, climate, and site factors controlling O_3 uptake with long-term growth. One of the important considerations in applying simulation modeling is to carefully assess the uncertainties associated with the modeling predictions. Further efforts need to be made to exercise the models so that they predict past growth losses associated with changes in O_3 exposures that can be verified with on-the-ground surveys.

AX9.4.6 Summary

A large number of studies pertinent to the development of exposure indices have been published since 1996, and these are predominantly focused on the development of a flux-based index to relate ambient O_3 to effects. There were only a few such studies prior to 1996 and these were reviewed in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996b). The few studies published since 1996 on the role of O_3 exposure components (including concentration, duration, and exposure patterns) in describing growth response to O_3 exposures have substantiated earlier conclusions of the importance of higher concentration, shape of the peak, and the episodicity of peak occurrence in the plant response to O_3 exposure. An inferred role of peak concentrations is possible from consideration of improved O_3 air quality in regions such as the San Bernardino Mountains in southern California. Studies provide the basis for focusing on the higher O_3 concentrations, while including the lower levels, when estimating the effects of emission reductions on vegetation.

A few studies have demonstrated the potential disconnection of the temporal patterns of peak events and maximal stomatal conductance. In addition, a few other studies have demonstrated the uptake of O_3 during nighttime hours, suggesting the need to cumulate O_3 exposure 24 h per day and not just during daylight hours.

Several studies since 1996 have demonstrated another critical concern in developing an index for exposure. The concern is that peak O_3 events and maximum stomatal conductance may be temporally separate. This disconnection introduces uncertainty in assessing O_3 impact when using the current ambient exposure based cumulative, concentration-weighted indices. If stomatal conductance is relatively low, as in the late afternoon in arid climates, and that is the same time as the peak O_3 concentrations, then use of an exposure index that does not consider this disconnect will overestimate the effect of the exposure. This concern is especially apparent when assessing the impact of O_3 across all the varied climatic regions of the United States or Europe. Some studies use stomatal models to predict uptake (Ashmore et al., 2004a) or physiological process-based models (Laurence et al., 2001) to integrate those species, climate, and site factors that drive this temporal pattern of stomatal conductance and exposure, and thus reduce some of the uncertainty in regional and national assessments of effects. These approaches, however, are still limited by being species-dependent.

The results of these studies and reviews indicate the need to continue to develop indices that are more physiologically and meteorologically connected to the actual dose of O_3 the plant receives. The cumulative concentration-weighted exposure indices are acknowledged surrogates for effective dose and are simple conceptually and easy to measure. They do not fully characterize the potential for plant uptake and resulting effects associated with O_3, because the indices, being measures of ambient concentration, do not include the physical, biological, and meteorological processes controlling the transfer of O_3 from the atmosphere through the leaf and into the leaf interior (U.S. Environmental Protection Agency, 1996b). Use of such indices is especially limited in spatial risk characterizations, because of the lack of linkage between meteorology and species- and site-specific factors influencing O_3 uptake. The flux-based approach should provide an opportunity to improve upon the concentration-based (i.e., exposure indices) approach. A cautionary argument was advanced in a few publications centered around the nonlinear relationship between O_3 uptake and plant injury (not growth alteration) response. The concern was that not all O_3 stomatal uptake results in a reduction in yield, which depends to some degree on the amount of internal detoxification occurring with each particular species; species having high amounts of detoxification potential may show less of a relationship between O_3 stomatal uptake and plant response.

The European approach and acceptance of flux-based critical values is a recognition of this problem; a concerted research effort is needed to develop the necessary experimental data and modeling tools that will provide the scientific basis for such critical levels for O_3 (Dämmgen et al., 1994; Fuhrer et al., 1997; Grünhage et al., 2004).

At this time, based on the current state of knowledge, exposure indices that differentially weight the higher hourly average O_3 concentrations but include the mid-level values represent the best approach for relating vegetation effects to O_3 exposure in the United States. A large database exists that has been used for establishing exposure-response relationships. Such a database does not yet exist for relating O_3 flux to growth response. The pattern disconnects between period of uptake and peak occurrence, as well as the potential for nocturnal uptake, should be considered by adding some weighting functions into the currently used exposure indices. Of particular consideration would be their inclusion in regional-to-national estimations of O_3 impacts on vegetation. Another useful approach to regional assessment for certain species

is to simulate growth effects with process-based models that account for seasonal climate and site factors that control conductance.

It is anticipated that, as the overlapping mathematical relationships of conductance, concentration, and defense mechanisms are better defined, O_3-flux-based models may be able to predict vegetation injury and/or damage at least for some categories of canopy-types with more accuracy than the exposure-response models.

AX9.5 OZONE EXPOSURE-PLANT RESPONSE RELATIONSHIPS

AX9.5.1 Introduction

Ambient O_3 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 O_3 exposure on vegetation remains a challenge. Numerous studies have related O_3 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 O_3 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 O_3 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 O_3 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 O_3. 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 O_3

research was conducted prior to the 1996 AQCD, the present discussion of vegetation response to O_3 exposure is largely based on the conclusions of the 1978, 1986, and 1996 criteria documents (U.S. Environmental Protection Agency, 1978, 1986, 1996). To provide a context for the discussion of recent research, the key findings and conclusions of those three documents are summarized below.

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

Experimental data reviewed in the 1978 and 1986 O_3 AQCDs dealt primarily with the effects of O_3 on agricultural crop species (U.S. Environmental Protection Agency, 1978, 1986). The chapter on vegetation effects in the 1978 O_3 AQCD (U.S. Environmental Protection Agency, 1978) emphasized foliar symptoms and growth effects, but not those effects that affected yield, an emphasis dictated by the kind of data available at the time. The 1986 O_3 AQCD reviewed a substantial new body of evidence based on OTC experiments (see Section AX9.1) showing that ambient O_3 exposures reduced the growth and yield of herbaceous plants, again with a focus on major crop species. In the 1986 and 1996 O_3 AQCDs, data were presented from regression studies conducted to develop exposure-response functions for estimating yield loss of major crop species in different regions of the United States. The 1996 O_3 AQCD included results from additional herbaceous crop species as well as shrub and tree species. For a number of tree species, biomass growth of seedlings was related to growing season O_3 exposures to produce response functions for estimating O_3 exposures that reduce growth by 10 or 30%. Also, in the 1986 and 1996 O_3 AQCDs, data from studies using EDU as a protectant were reviewed. The 1978, 1986, and 1996 O_3 AQCDs also reviewed data on the response to O_3 exposures of forest ecosystems in the San Bernardino Mountains of southern California (U.S. Environmental Protection Agency, 1978, 1986, 1996). Because this region is exposed to high concentrations of O_3 and has shown evidence of ecosystem-level changes, it remains an important study area (see Section AX9.6).

Ozone can cause a range of effects, beginning with individual cells, leaves, and plants, and proceeding to plant populations and communities. These effects may be classified as either "injury" or "damage". Injury encompasses all plant reactions, such as reversible changes in plant metabolism (e.g., altered photosynthetic rate), altered plant quality, or reduced growth that

does not impair yield or the intended use or value of the plant (Guderian, 1977). In contrast, damage includes all effects that reduce or impair the intended use or value of the plant. Damage includes reductions in aesthetic values as well as losses in terms of weight, number, or size of the plant part that is harvested (yield loss). Yield loss also may include changes in crop quality, i.e., physical appearance, chemical composition, or the ability to withstand storage. Losses in aesthetic values are difficult to quantify. Although foliar symptoms cannot always be classified as damage, their occurrence indicates that phytotoxic concentrations of O_3 are present, and, therefore, studies should be conducted to assess the risk to vegetation.

Visible symptoms due to O_3 exposures reduce the market value of certain crops and ornamentals for which leaves are the product, e.g., spinach, petunia, geranium, and poinsettia. The concept of limiting values used to summarize foliar symptoms in the 1978 O_3 AQCD (U.S. Environmental Protection Agency, 1978) was also considered valid in the 1986 O_3 AQCD (U.S. Environmental Protection Agency, 1986). Jacobson (1977) developed limiting values by assessing the available scientific literature and identifying the lowest exposure concentration/duration reported to cause foliar symptoms in a variety of plant species. Graphical analyses presented in those documents indicated that the limit for reduced plant performance was an exposure to 50 ppb for several hours per day for more than 16 days. Decreasing the exposure period to 10 days increased the concentration required to cause symptoms to 100 ppb; and a short, 6-day exposure further increased the concentration required to cause symptoms to 300 ppb. These limiting values established in 1978 were still deemed appropriate in the 1986 and 1996 O_3 AQCDs. Such foliar symptoms are caused by O_3 concentrations that occur in the United States as shown in Table AX9-13 (adapted from U.S. Environmental Protection Agency, (U.S. Environmental Protection Agency, 1996).

The 1986 O_3 AQCD emphasized that, although foliar symptoms on vegetation are often an early and obvious manifestation of O_3 exposure, O_3 effects are not limited to foliar symptoms. Other effects include reduced growth of many organs (including roots), changes in crop quality, and alterations in plant susceptibility to biotic stressors and sensitivity to abiotic stressors. The 1986 O_3 AQCD also emphasized that O_3 exerts phytotoxic effects only if a sufficient amount of O_3 reaches sensitive sites within the leaf (Section AX9.2). Ozone injury will not occur if the rate of O_3 uptake is low enough that the plant can detoxify or metabolize O_3 or its metabolites or if the plant is able to repair or compensate for the effects (Tingey and Taylor, 1982; U.S.

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

3-Month Growing Season (June-August)

Year	No. of Sites[b]	2HDM[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%
Among Years		0.113	11.1%	0.05	10.0%	87.0	9.9%	29.5	42.1%	29.4	31.0%

5-Month Growing Season (May-September)

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%
Among Years		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 O_3 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).

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

Beginning in the 1986 O_3 AQCD and continuing in the 1996 O_3 AQCD, OTC studies that better quantified the relationship between O_3 exposure and effects on crop species were reviewed, with a focus on yield loss. These studies can be grouped into two types, depending on the experimental design and statistical methods used to analyze the data: (1) studies that developed predictive equations relating O_3 exposure to plant response, and (2) studies that compared the effects of discrete treatment level(s) to a control. The advantage of the regression approach is that exposure-response models can be used to interpolate results between treatment levels.

Discrete treatment experiments were designed to test whether specific O_3 treatments were different from the control rather than to develop exposure-response equations, and the data were analyzed using analyses of variance. When summarizing these studies using discrete treatment levels, the lowest O_3 concentration that significantly reduced yield was determined from analyses done by the original authors. Often, the lowest concentration used in a study was the lowest concentration reported to reduce yield; hence, it was not always possible to estimate a no-effect exposure concentration. In general, the data indicated that 100 ppb O_3 (frequently the lowest concentration used in the studies) for a few hours per day for several days to several weeks usually caused significant yield reductions of 10 to 50%.

By the time the 1986 O_3 AQCD was prepared, much new information concerning the effects of O_3 on the yield of crop plants had become available through EPA's NCLAN research program and through research funded by other agencies. The NCLAN project was initiated by the EPA in 1980 primarily to improve estimates of yield loss under field conditions and to estimate the magnitude of crop losses caused by O_3 throughout the United States (Heck et al., 1982; Heck et al., 1991). The cultural conditions used in the NCLAN studies approximated typical agronomic practices. The primary objectives were:

(1) to define relationships between yields of major agricultural crops and O_3 exposure as required to provide data necessary for economic assessments and development of O_3 NAAQS;

(2) to assess the national economic consequences resulting from O_3 exposure of major agricultural crops; and

(3) to advance understanding of cause-and-effect relationships that determine crop responses to pollutant exposures.

Using NCLAN data, the O_3 concentrations predicted to cause 10 or 30% yield loss were estimated using linear or Weibull response functions. The data in Table AX9-14 are from the 1996 document and were based on yield-response functions for 38 species or cultivars developed from studies using OTCs of the type developed by Heagle et al. (1973) (see Section AX9.1). Composite exposure-response functions for both crops and tree seedlings as a function of O_3 exposure expressed as SUM06 are shown in Figure AX9-19. Review of these data indicate that 10% yield reductions could be predicted for more than 50% of experimental cases when: (1) 12-h SUM06 values exceeded 24.4 ppm·h, (2) SIGMOID values exceeded 21.5 ppm·h, or (3) 7-h seasonal mean concentrations were 50 ppb. The SIGMOID index is very similar to the W126 index (see Section AX9.4 for further information about O_3 indices). Much lower values are required for each index to protect 75% of experimental cases (Table AX9-14). Grain crops were generally found to be less sensitive than other crops. The data summarized in the 1996 criteria document also indicated that the variation in sensitivity within species may be as great as differences between species.

The chemical protectant, EDU, was also used to provide estimates of yield loss. The impact of O_3 on yield was determined by comparing the yield data from plots treated with EDU versus untreated plots. Studies indicated that yields were reduced by 18 to 41% when daytime ambient O_3 concentrations exceeded 80 ppb for 5 to 18 days over the growing season. For this approach to be credible, the effects of EDU itself on a particular species must be preestablished under conditions without O_3 exposure (Kostka-Rick and Manning, 1992a).

The 1996 O_3 AQCD reviewed several experiments demonstrating that the seedlings of some tree species such as poplars (*Populus* spp.) and black cherry are as sensitive to O_3 as are annual plants, in spite of the fact that trees are longer-lived and generally have lower rates of gas exchange, and, therefore, a lower uptake of O_3. The 1996 document also reviewed data showing that O_3 exposures that occur at present in the United States are sufficient to affect the growth of a number of trees species. For example, exposure-response functions for 51 cases of tree seedling responses to O_3, including 11 species representing deciduous and evergreen growth habits, suggest that a SUM06 exposure for 5 months of 31.5 ppm·h would protect hardwoods from a 10% growth loss in 50% of the cases studied (Table AX9-15). Similarly, a SUM06 exposure of

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]

50th PERCENTILE[b]	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
75th PERCENTILE[b]								
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

[a]See Appendix A for abbreviations and acronyms.
[b]The numbers in parentheses are the number of cases used in deriving the various exposure levels.
[c]Standard error (SE).
[d]NCLAN data refers to studies conducted as part of the NCLAN project. Wet and dry refer to watering regimes used in the studies, wet being well-watered, and dry meaning some level of drought stress was imposed.
[e]24-h exposure statistics reported in Lee et al. (1994b). Relative yield loss for 2HDM is relative to yield at 40 ppb rather than 0 ppb as was used in Tingey et al. (1991).

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

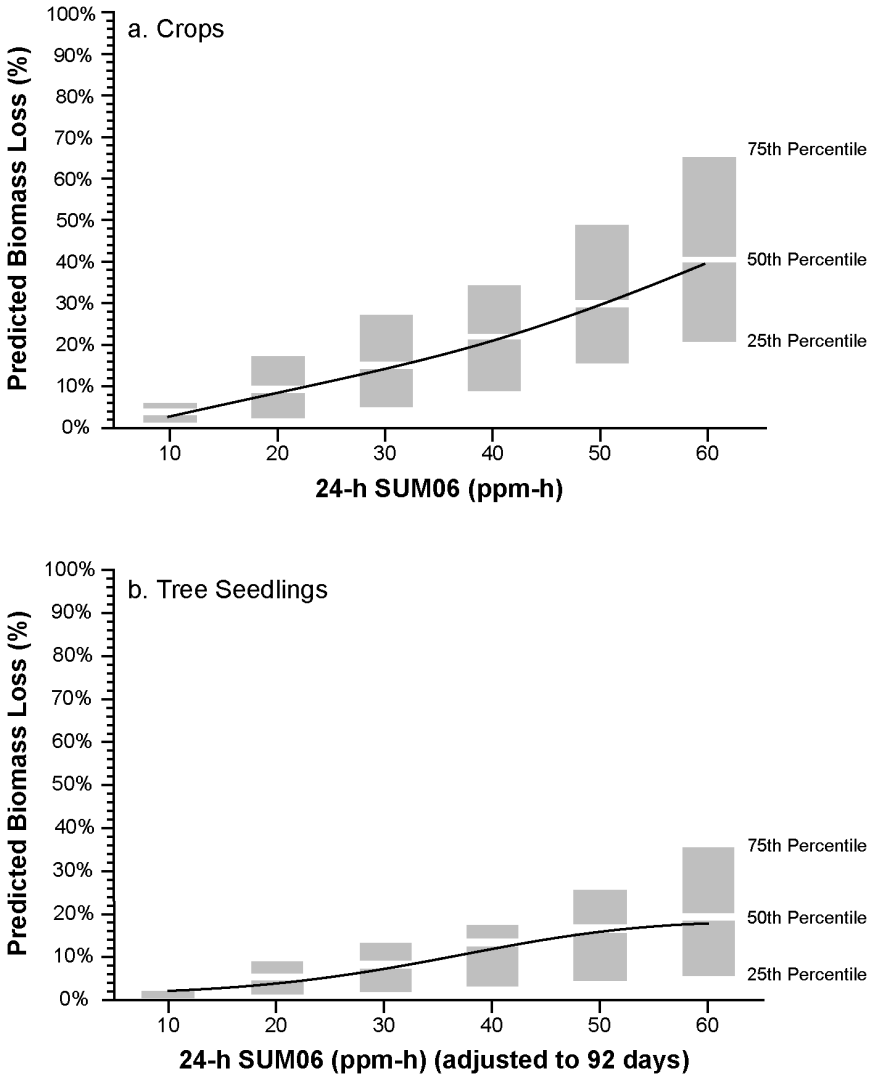


Figure AX9-19. Distribution of biomass loss predictions from Weibull and linear exposure-response models that relate biomass to O_3 exposure. Exposure is characterized with the 24-h SUM06 statistic using data from (a) 31 crop studies from National Crop Loss Assessment Network (NCLAN) and (b) 26 tree seedling studies conducted at U.S. Environmental Protection Agency's Environmental Research Laboratory in Corvallis, OR; Smoky Mountains National Park, TN; Houghton, Michigan; and Delaware, Ohio. Separate regressions were calculated for studies with multiple harvests or cultivars, resulting in a total of 54 individual equations from the 31 NCLAN studies and 56 equations from the 26 seedling studies. Each equation was used to calculate the predicted relative yield or biomass loss at 10, 20, 30, 40, 50, and 60 ppm·h, and the distributions of the resulting loss were plotted. The solid line is the calculated Weibull fit at the 50th percentile.

Source: U.S. Environmental Protection Agency (1996); Hogsett et al. (1995).

Table AX9-15. SUM06 Levels Associated with 10 and 20% Total Biomass Loss for 50 and 75% of the Seedling Studies (The SUM06 value is adjusted to an exposure length of 92 days per year.)[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

[a]See Appendix A for abbreviations and acronyms.
[b] PRYL = predicted relative yield (biomass) loss

Source: U.S. Environmental Protection Agency (1996), based on Hogsett et al. (1995).

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

In 1986, the EPA (U.S. Environmental Protection Agency, 1986) established that 7-h per day growing season mean exposures to O_3 concentrations above 50 ppb were likely to cause measurable yield loss in agricultural crops. At that time, few conclusions could be drawn about the response of deciduous or evergreen trees or shrubs, due to the lack of information about

response of such plants to season-long exposures to O_3 concentrations of 40 to 60 ppb and above. However, the 1978 and 1986 O_3 AQCDs (U.S. Environmental Protection Agency, 1978, 1986) indicated that the limiting value for foliar symptoms on trees and shrubs was 60 to 100 ppb for 4 h. From 1986 to 1996, extensive research was conducted, establishing the sensitivity of many tree species. Based on research published since the 1986 O_3 AQCD (U.S. Environmental Protection Agency, 1986), a number of conclusions were drawn in 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996):

(1) An analysis of 10 years of monitoring data from more than 80 to almost 200 nonurban sites in the United States established ambient 7-h growing season average concentrations of O_3 for 3 or 5 months of 51 to 60 ppb and 47 to 54 ppb, respectively. The SUM06 exposures ranged (a) from 24.8 to 45.2 ppm·h for 3 months and (b) from 32.7 to 58.0 ppm·h for 5 months (Tingey et al. (1991), Table AX9-13).

(2) The results of OTC studies that compared yields at ambient O_3 exposures with those in filtered air and retrospective analyses of crop data (Table AX9-14) established that ambient O_3 concentrations were sufficient to reduce the yield of major crops in the United States. Research results since 1978 did not invalidate EPA conclusions (U.S. Environmental Protection Agency, 1978, 1986) that foliar symptoms due to O_3 exposures reduce the market value of certain crops and ornamentals where leaves are the product (such as spinach, petunia, geranium, and poinsettia) and that such damage occurs at ambient O_3 concentrations observed in the United States.

(3) A 3-month SUM06 exposure of 24.4 ppm·h, corresponding to a 7-h mean of 49 ppb and a 2HDM of 94 ppb O_3 may prevent a 10% loss in 50% of the 49 experimental cases analyzed by Tingey et al. (1991). A 12-h growing season mean of 0.045 ppb should restrict yield losses to 10% in major crop species (Lesser et al., 1990).

(4) Depending on duration, concentrations of O_3 and SUM06 exposures currently in the United States are sufficient to affect the growth of a number of tree species. Given the fact that multiple-year exposures may cause a cumulative effect on the growth of some trees (Simini et al., 1992; Temple et al., 1992), it is likely that a number of species currently are being impacted, even at ambient O_3 exposures (Tables AX9-13 and AX9-20).

(5) Exposure-response functions for 51 cases of seedling response to O_3 (Hogsett et al., 1995), including 11 species representing deciduous and evergreen growth habits, suggest that a SUM06 exposure for 5 months of 31.5 ppm·h would protect hardwoods from a 10% growth loss in 50% of the cases studied. A SUM06 exposure of 42.6 ppm·h should provide the same level of protection for evergreen seedlings. Note that these conclusions do not take into the account the possibility of effects on growth in subsequent years, an important consideration in the case of long-lived species.

(6) Studies of the response of trees to O_3 have established that, in some cases (for instance, poplars and black cherry), trees are as sensitive to O_3 as are annual plants, in spite of the fact that trees are longer-lived and generally have lower gas exchange rates, and, therefore, lower O_3 uptake.

(7) Use of the chemical protectant, EDU, is of value in estimating O_3-related losses in crop yield and tree growth, provided that care is exercised in establishing appropriate EDU dosages to protect the plants without affecting growth.

The major question to be addressed in the remainder of this section is whether new information supports or alters the 1996 criteria document conclusions summarized above. In particular, this section evaluates whether the response of plants to experimental treatments at or near O_3 concentrations characteristic of ambient levels in many areas of the United States (Tables AX9-13 and AX9-20) can be compared to a control or reduced O_3 treatment to establish a potential adverse effect. Before evaluating new information from the literature on O_3 effects on vegetation, O_3 exposure indices used in O_3 studies and trends in O_3 exposure patterns during the past two decades are briefly reviewed.

AX9.5.3 Ozone Indices and Ambient Exposure

As recognized in both the 1986 and the 1996 criteria documents, the characterization and representation of the exposure of vegetation to O_3 is problematic, because the specific aspects of pollutant exposure that cause injury or damage are difficult to quantify. This issue was addressed in Section AX9.4, and only a few points will be discussed here in order to provide a context for interpreting data on exposure-response relationships. The most important effects of O_3 on vegetation occur due to uptake of O_3 through stomata, with subsequent oxidative injury that appears to be rather nonspecific (Section AX9.2). As has been discussed by numerous authors during the last three decades, from a toxicological and physiological view, it is much more realistic to relate effects to internal (absorbed) O_3 dose rather than to exposure near the leaf or canopy (Fowler and Cape, 1982; Fuhrer et al., 1992; Grünhage et al., 1993, 1999; Legge et al., 1995; Massman et al., 2000; Musselman and Massman, 1999; Pleijel et al., 1995b; Runeckles, 1974; Taylor et al., 1982; Tingey and Taylor, 1982) (see also Section AX9.4). Theoretically, flux estimates should improve the assessment of O_3 effects, but despite recent attention to this topic, particularly in Europe, it remains difficult to estimate flux in the field outside of

experimental sites where continuous measurements of wind speed and other environmental conditions are made. This topic is discussed further below in Section AX9.5.4.5.

No simple exposure index can accurately represent all of the numerous factors operating at different timescales that affect O_3 flux into plants and subsequent plant response (Section AX9.4). Indices of peaks, such as the 2HDM, are not well suited for discerning exposure-response relationships, because they do not capture the effects of lower O_3 concentrations nor the cumulative effects of O_3 on vegetation (Heck and Cowling, 1997; U.S. Environmental Protection Agency, 1996). Therefore, peak indices have not been used in recent decades to develop exposure-response relationships for vegetation. Fortunately, other simple indices have shown substantial correlation with responses such as crop yield under experimental conditions. During the 1980s, the most commonly used indices for expressing O_3 exposure were 7-, 8-, or 12-h daytime average values over the duration of O_3 exposure, which was often 3 months or somewhat less for experimental studies with crops. These indices perform reasonably well for interpreting experimental data on the response of vegetation to O_3, particularly for individual experiments, although they do not explain all of the variation among experiments in retrospective analysis of multiple experiments (Lesser et al., 1990).

Since the 1980s, cumulative indices such as the SUM06, AOT40, or W126 that preferentially weight higher concentrations have been used in conjunction with mean indices for developing exposure-response relationships (Tables AX9-14 and AX9-15, and Figure AX9-18). Such indices are often more suitable than mean values, because they are cumulative and because they preferentially weight higher concentrations. Thus, these indices generally provide somewhat better fits to experimental data than do mean indices, especially in retrospective analyses of multiple experiments on multiple species (Lee et al., 1994a; Lee et al., 1994b; Lee and Hogsett, 1999; Tingey et al., 1991). Unfortunately, no single index has been used consistently even in the recent literature, making it difficult to compare results among and between experiments and with ambient exposure data. However, Tables AX9-13 and AX9-20 provide summaries of ambient exposure data for several indices that can be compared to the experimental results reviewed in the remainder of this section. Of the cumulative indices that preferentially weight higher concentrations, the SUM06 index has been used most commonly in the U.S. literature, and it was selected in a meeting of scientific experts on O_3 effects on vegetation as suitable for a secondary standard to protect vegetation (Heck and Cowling, 1997).

However, it should be noted that the W126 index has been selected for use in protecting vegetation in Class 1 areas (Federal Land Manager's Air Quality Related Values Workgroup (FLAG), 2000). Even in recent studies, O_3 data are often presented using only a seasonal mean index value, and so mean values are frequently presented in this section. Such reporting of mean indices should not be interpreted as a preference for them, but rather as a limitation in the data reported in the literature. Additional information about O_3 exposure for individual experiments, including the number and type of O_3 treatments (addition of a constant concentration of O_3 or an amount proportional to ambient levels), and duration, are reported in Tables AX9-16 through AX9-19.

Since the 1996 O_3 AQCD, the use of the AOT40 index has become quite common in Europe for identifying and mapping areas of exceedance, but it has not been used much in the United States. Thus, studies reporting O_3 exposure only as AOT40 values are presented in tables summarizing effects on annual, herbaceous perennial, and woody vegetation. However, such studies are not as commonly cited in the text of this section, because AOT40 summary data on O_3 exposures in the United States are rarely available. This lack makes it difficult to compare experimentally derived exposure-response data expressed as AOT40 to ambient U.S. O_3 exposures. The development of critical levels in Europe has been based primarily on the AOT40 index, so this index is discussed in that context.

In addition to peak weighting, there is also evidence that the timing of exposure during plant growth is important. For example, the greatest effects on grain yield are due to exposure during grain filling, rather than earlier or later in the growing season (Lee et al., 1988; Pleijel et al., 1998; Soja et al., 2000; U.S. Environmental Protection Agency, 1996; Younglove et al., 1994). A recent study grew bush bean in OTCs with CF or above-ambient O_3 using exposure dynamics typical of the Midwestern United States for three time periods: (1) the entire season, (2) the period prior to anthesis, (3) during pod filling and maturation (Tingey et al., 2002). Ozone exposure prior to anthesis reduced growth by less than 1% per ppm·h (SUM06) while exposure during pod filling and maturation reduced growth by 4 to 7%. A meta-analysis of 53 studies of O_3 effects on soybean found that O_3 had greater effects with increases in developmental stage, with the greatest effect during seed filling (Morgan et al., 2003). The importance of respite times was discussed in the previous criteria documents (U.S. Environmental Protection Agency, 1978, 1986, 1996) but remains difficult to quantify

Table 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 year	Pod dry weight	n.s. for Tenderette, 90 for S156	Heagle et al. (1999b)
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. (1999b)
Corn	OTC	Beltsville, MD	CF, +40: 7-h mean = 20, 70	1 year	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 year	Seed-cotton weight	22	Heagle et al. (1999b)
Cotton, cv. Deltapine	OTC	Raleigh, NC	CF, NF, 1.5×, 12-h mean = 24, **51**, 78	1 year	Seed-cotton weight	21, 49 (NF, 1.5×)	Heagle et al. (1999b)
Oat, cv. Vital	OTC	Ostad, Sweden	CF, NF: 7-h mean = 12, **27**	1 year	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 year	Tuber yield	15, 31 for Norland in NF and 1.5x; 11 for Superior in 1.5x	Heagle et al. (1999b)
Potato[2]	**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, +O3: 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. (1998b)
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. (1998b)
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. (1998b)
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. (1998b)
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)
Soybean, cv. 93B15	FACE	Urbana-Champaign, IL	AA, +O3 in 2002 8-h mean = **62**, 75; SUM06 = **36**, 70 ppm•h; AOT40 = **22**, 42 ppm•h; in 2003 8-h mean = **50**, 63; SUM06 = **22**, 53 ppm•h; AOT40 = **14**, 34 ppm•h	2 expts of 1 year	Seed yield	15, 25 (2002, 2003)	Morgan et al. (in press)
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)
Wheat[1], 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)**	**13 studies of 1 year each**	**Grain yield**	**13 (n.s.)**	Bender et al. (1999)
			AOT40 mean (SD) low = 6.18 (8.54) ppm•h, AOT40 mean (SD) high = 28.23 (23.05) ppm•h				Hertstein et al. (1999)

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[1]	**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 days/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 days/week, 7 weeks	Grain yield	+17	Finnan et al. (1996)
Wheat, cv. Promessa	OTC	SE Ireland	CF, +25, +50: 12-h total = 6.7, 34, 34 ppm•h	+25 = 6 h/day, 5 days/week, +50 = 3 h/day, 5 days/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)

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. 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 (*Capillaris* 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)
Lespedeza, *Sericea*	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)
Phleum alpinum	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 (1999a)
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. (2000c)

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 m^2	7 episodes of 5 days	Biomass, diameter	No effect	Bortier et al. (2001b)
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. (2003b)
Birch, [*B. pubescens*]	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; 4 y 1.5x 12-h mean = 54.5, 51.1, 48.9, 52.8	7 years (only 4 years of O_3 data reported)	Volume (d2*h)	No effect	Isebrands et al. (2001)
Cherry, black	2 years	OTC	Norris, TN	CF, 1×, 2×: 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 (*P. tremuloides* × *P. tremula*)	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. (1998a)
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. (2000b)
Pine, Scots	Seedling	OTC	Switerzland	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. (2000b)
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	Switerzland	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	Switzerland	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	Sapling	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.

(Section AX9.4). Even when some of these aspects of O_3 exposure can be elucidated, it is difficult to apply this knowledge to developing exposure-response relationships based on data in the scientific literature, because O_3 exposure is often reported only in the form of a summary index such as a 12- or 24-h mean, SUM06, or AOT40.

Table AX9-13 presents summaries of ambient O_3 exposure patterns in the United States for 1982 to 1991 for several indices including the 7-h mean and SUM06. More recent summaries for the entire United States for these indices are not available, but Table AX9-20 summarizes more recent data for the central and eastern United States. As shown in Table AX9-20, from 1989 to 1995, mean 12-h 3-month SUM06 values (in ppm·h) at 41 rural sites in the Clean Air Status and Trends Network were 31.5 for the Midwest, 18.9 for the Upper Midwest, 33.2 for the Northeast, 13.2 for the Upper Northeast (New Hampshire, Maine), 34.5 for the South-Central, and 19.2 for the Southern Peripheral subregions (Baumgardner and Edgerton, 1998). These results are important because these sites were selected to represent rural areas, while many other monitoring sites represent urban or suburban areas. For these same subregions, W126 values ranged from 12.8 to 25.6 ppm·h. From 1989 to 1995, O_3 concentrations decreased about 5% for daily and 7% for weekly values for most of these sites, after adjusting for meteorological conditions (Holland et al., 1999). These trends were statistically significant at about 50% of the sites ($p \leq 0.05$). However, because the trend analysis was intended to examine the efficacy of O_3 emissions controls, the trends were adjusted for meteorological conditions. Thus, they do not reflect the actual trends in O_3 exposure over time.

AX9.5.4 Effects of Ozone on Annual and Biennial Species

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

An extensive search of the literature was performed using several electronic databases to identify scientific articles containing quantitative information on both the amount of O_3 exposure and its effects on vegetation. Greater emphasis is placed on studies with longer duration with O_3 exposure concentrations and environmental conditions that were as similar as possible to ambient conditions. Many of the studies reviewed herein were conducted in OTCs. In the United States, nearly all of such studies have used the type of OTC developed by Heagle et al.

Table AX9-20. Ozone Exposures at 35 Rural Sites in the Clean Air Status and Trends Network in the Central and Eastern United States From 1989 to 1995

Subregion	SUM06 12-h, 3-Month Mean	SUM06 12-h, 3-Month SD	W126 3-Month Mean	W126 3-Month SD	Max. 8 h > 80 ppb(n) Mean	Max. 8 h > 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).

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

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

Most research on the effects of O_3 on herbaceous species has evaluated growth, biomass, or yield of commercial portions of crop or forage species. It is well established that reproductive organs such as seeds may be particularly sensitive to injury or biomass reductions due to O_3, as reviewed by (Black et al., 2000). As discussed in Section AX9.3, numerous analyses of

experiments conducted in OTCs and with naturally occurring gradients demonstrate that the effects of O_3 exposure vary depending on the growth stage of the plant. Plants grown for seed or grain are often most sensitive to exposure during the seed or grain-filling period (Lee et al., 1988; Pleijel et al., 1998; Soja et al., 2000; Younglove et al., 1994), whereas plants grown for biomass production, such as alfalfa, may be sensitive throughout the growth period (Younglove et al., 1994). However, because different species are sensitive during different periods of their growth and, because planting or germination dates vary throughout large regions even for a single species, no single phenological weighting scheme can appropriately and practically represent all vegetation in all locations in the United States. For natural populations, reductions in seed yield might be particularly important if subsequent seedling establishment is compromised by O_3.

Green beans (cv. Pros) were grown in pots in OTCs in the Netherlands for 62 days and exposed to 6 treatments consisting of constant O_3 additions to CF chambers (see Section AX9.1) for 9 h/day (Tonneijck and Van Dijk, 1998). Bean yield response to O_3 was nonlinear, with an apparent threshold near the CF30 (charcoal filtered with a constant addition of 30 ppb O_3) treatment with a 9-h mean O_3 concentration of 28 ppb and an AOT40 value of 0.1 ppm·h). Yield was reduced by 29% at a 9-h mean value of 44 ppb corresponding to an AOT40 value of 3.6 ppm·h (Table AX9-16). Beans were grown in pots in OTCs for 3 months with the following O_3 treatments: CF, nonfiltered (NF), CF with O_3 added up to ambient, and CF with 2×-ambient O_3 (Brunschon-Harti et al., 1995). Ozone reduced pod mass by 56% with a mean concentration of 32 ppb in the 2× ambient treatment as compared with 1 ppb in the CF treatment (daily averaging time not reported). A second treatment factor in this experiment was addition of EDU, as discussed below under the heading "Studies Using Ethylene Diurea as a Protectant." Bush beans (cv Bush Blue Lake 290) were grown in pots in OTCs in Corvallis Oregon for 63 or 65 days in two experiments, one from May to July and one from August to October (Tingey et al., 2002). Plants were exposed to either CF air or CF air with above-ambient O_3 with temporal frequency and exposure dynamics typical of the Midwestern United States, with SUM06 values of 0.0 for the CF treatment and 75.7 or 68.4 ppm·h for the two experiments. Ozone exposure reduced pod dry weight by 51 and 57% in the two experiments. The sensitive cultivar S156 and the more resistant cultivar Tenderette were grown in pots in OTCs in Raleigh, NC at either CF (12-h mean of 23 ppb) or 1.4× (12-h mean of 72 ppb) (Heagle et al., 2002). At final harvest, the dry weight

of the sensitive cultivar was reduced 90% by the O_3 treatment, but the more resistant cultivar was not reduced. In two additional OTC experiments during 2000 and 2001 in Raleigh, NC, the sensitive cultivar S156, the moderately sensitive cultivar Oregon-91, and the more resistant cultivar R123 were grown in CF, NF, and ambient air treatments (Fiscus et al., 2005). For the NF treatment of the two experiments, the yield of S156 was reduced 21% and 45%, that of Oregon-91 by 27% only in 2001, while R123 was not significantly reduced. These yield reductions are greater than those previously reported in four similar studies summarized in the 1996 AQCD (Table 5-25 of U.S. Environmental Protection Agency, 1996). Greater sensitivity in the more recent experiments may be due to cultivar differences or other differences in experimental protocols.

In a study with OTCs on silty loam soil in Beltsville, MD, corn yield was reduced by 13% with exposure to a 7-h mean concentration of 70 ppb O_3 compared to a CF treatment with a 7-h mean concentration of 20 ppb (Mulchi et al., 1995; Rudorff et al., 1996a,c). In this study, different amounts of O_3 were added above ambient levels for 5 days follows: 20, 30, 40, 50, 60 ppb, except that O_3 was not added to exceed a total concentration of 120 ppb (Rudorff et al., 1996a,c).

In two studies conducted in Raleigh, NC, cotton (cv. Deltapine 51) was grown in pots and exposed to CF and 1.5× (nonfiltered, see Section AX9.1) O_3 in one year, and CF, NF, and 1.5×-ambient O_3 in the second year, with ambient and elevated CO_2 concentrations (Heagle et al., 1999b) (Table AX9-16). In the first year, yield decreased by 22% with 1.5×-ambient O_3 (12-h mean value of 71 ppb). In the second year, yield decreased by 21% and 49% with exposure to ambient or 1.5× ambient O_3 (12-h mean values of 51 and 78 ppb, Table AX9-16). Increased CO_2 levels prevented or reduced this yield suppression (Heagle et al., 1999b). These yield reductions are similar to those reported previously in four similar studies summarized in the 1996 AQCD (Table 5-25 of U.S. Environmental Protection Agency, 1996).

In a study of oats in OTCs in southern Sweden, exposure to ambient (NF) O_3 did not affect grain yield (Pleijel et al., 1994a). Ambient O_3 concentration expressed as a 7-h mean was 27 ppb, with only 1 h greater than 80 ppb and none above 90 ppb.

The interactive effects of elevated O_3 and CO_2 additions on potato yield (cv Bintje) were studied in OTCs at 6 sites in northern Europe (Craigon et al., 2002). Ozone was added to a target daily average value of 60 ppb, and AOT40 values across all years and experiments ranged

from ~6 to 27 ppm·h. The O_3 treatment reduced total tuber yield an average of 4.8% with elevated O_3 treatment across all experiments (Craigon et al., 2002). This total effect was statistically significant even though the effects of individual experiments generally were not (Craigon et al., 2002), due to the increased statistical power of the pooled analysis. Several publications report other aspects of the "CHIP" experiments or present results of individual experiments (De Temmerman et al., 2002a,b; Donnelly et al., 2001a,b; Fangmeier et al., 2002; Finnan et al., 2002; Hacour et al., 2002; Lawson et al., 2002; Pleijel et al., 2002; Vandermeiren et al., 2002; Vorne et al., 2002). Resistant and susceptible cultivars of potato (Superior and Dark Red Norland, respectively) were grown for one season in Raleigh, NC and exposed to CF, NF, and 1.5× ambient O_3 treatments with 12-h mean values of 15, 45, 80 (Heagle et al., 2003). Tuber yield was decreased by 15 and 31% for Dark Red Norland in NF and 1.5x treatments, but by only 11% in only the 1.5x treatment for Superior.

The effect of an intermittent constant addition of O_3 using a free air exposure system in Northumberland, UK was investigated with the oilseed rape cultivar Eurol (Ollerenshaw et al., 1999). Ozone was added for 6 h/day for 17 days. The ambient treatment had a mean value of 30 ppb and the O_3 addition treatment had a mean of 77 ppb. After overwintering, O_3 was added for 32 days for 7 h/day between May and June (mean values of 31 and 80 ppb). Yield was reduced by 14% despite the lack of any foliar symptoms.

Field fumigation chambers ventilated with fans on both ends were used to assess effects of five O_3 treatments on rice over 3 years in Japan (Kobayashi et al., 1994, 1995). All O_3 treatments used CF air, and O_3 was added to the 0.5, 1.0, 1.5, 2.0, or 2.75× ambient concentration for 7 h/day. Based on a linear regression for the 3 years, yield decreased by 3 to 10% at a 7-h mean concentration of 40 ppb (Table AX9-16). This decrease is greater than that found for rice in earlier studies in California (Kats et al., 1985b), although whether this difference is due to differences in cultivars, experimental treatment, or environmental factors cannot be determined.

During 3 years in Beltsville, MD, the soybean cultivars Essex and Forrest were exposed to CF air and NF air in OTCs (Chernikova et al., 2000; Robinson and Britz, 2000) with O_3 added as described for experiments with corn and wheat at Beltsville (Mulchi et al., 1995; Rudorff et al., 1996c). During 1994 and 1995, as previously found for these cultivars, Essex was less sensitive than Forrest, with yield decreases of 10% (n.s.: $p > 0.1$) compared to 32% for Forrest ($p < 0.01$)

(Chernikova et al., 2000). There was no evidence of water stress in this experiment. In 1997, the two O_3 treatments were CF (7-h mean = 24 ppb) and NF with a constant addition of O_3 (7-h mean = 58 ppb) (Robinson and Britz, 2000). The yield of Essex was not significantly affected, while the yield of Forrest was decreased by 21% (Table AX9-16).

In a study in Raleigh, NC, the soybean cultivar Essex was grown in pots and exposed to CF and 1.5× ambient O_3 concentrations during three growing seasons (Fiscus et al., 1997). Over the 3 years, exposure to an average 12-h mean O_3 concentration of 82 ppb reduced soybean yield by 41% (Table AX9-16). In similar studies also in Raleigh, NC, Essex was exposed to CF, NF, and 1.5× ambient O_3 for two seasons (Heagle et al., 1998b). Yield decreased by 16% and 15% in the 2 years by ambient O_3 (12-h mean values of 50 and 42 ppb), and decreased by 37 and 40% with exposure to 1.5× ambient O_3 (12-h mean values of 79 and 69 ppb, Table AX9-16). In this same experiment in the second year, similar yield reductions were observed for the cultivar Holladay, while the growth of cultivar NK-6955 was increased substantially by ambient O_3 exposure. All three cultivars were grown in the same chambers in this experiment, and the authors suggested that NK-6955 plants may have shaded the other cultivars to some extent.

In a 2-year study using OTCs in Raleigh, NC, the soybean cultivars Coker 6955, Essex, and S53-34 were exposed to CF, NF, and 1.5× ambient O_3 treatments (Miller et al., 1994). Seasonal mean 12-h O_3 concentrations ranged from 14 to 83 ppb. As compared to the CF treatment, ambient O_3 exposure (NF treatment) reduced seed yield by 11 to 18% except for Coker 6955 in the first year (1989), which showed a yield increase of 14%. The 1.5× ambient O_3 treatment reduced yield by 32 to 56% in all cultivars in both years. In a similar subsequent experiment with the cultivar Essex, exposure to a 12-h mean ambient O_3 concentration of 50 ppb reduced yield by 11%, while exposure to 79 ppb reduced yield by 22% (Table AX9-16) (Miller et al., 1998). In similar experiments with Essex during 1999 and 2000, plants were exposed to 1.5× ambient O_3 in OTCs either in pots or planted in the ground (Booker et al., 2005). Exposure to a 12-h mean ambient O_3 concentration of 75 ppb reduced yield by 27% in pots and 24% in the ground in 1999, and exposure to a 12-h mean ambient O_3 concentration of 67 ppb reduced yield by 41% in pots and 39% in the ground in 2000 (differences between results in pots and in the ground were not statistically significant).

In a study using a free air exposure system near Urbana-Champaign, IL, the soybean cultivar 93B15 was grown for two seasons (2002, 2003) and exposed to ambient ozone and

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

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

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

During the 1990s, a major European research program investigated the combined effects of CO_2, O_3, and other physiological stresses on wheat (Bender et al., 1999; Hertstein et al., 1996,

1999; Jäger et al., 1999). The ESPACE-wheat program included 13 experiments in OTCs at eight sites in northern Europe over 3 years. Low- and high-O_3 exposures in these experiments had the following values: 12-h mean (SD) low = 26.3 ppb (12.2), 12-h mean (SD) high = 51.37 (18.3) ppb, AOT40 mean (SD) low = 6.2 (8,5) ppm·h, AOT40 mean (SD) high = 28.3 (23.0) ppm·h, as calculated from data presented in Table 3 of Hertstein et al. (1999). An analysis of all 13 experiments showed that high O_3 at ambient CO_2 reduced yield by 13% on average (Bender et al., 1999). However, this reduction was not statistically significant based on an ANOVA, and the authors concluded that the wheat cultivar Minaret may be relatively tolerant to O_3 (Bender et al., 1999). Results of some individual studies within this program have been reported previously (Donnelly et al., 1999; Fangmeier et al., 1996, 1999; Mulholland et al., 1997a, 1998a,b; Pleijel et al., 2000b).

In a study with OTCs on silty loam soil in Beltsville, MD, wheat yield was reduced by 20% on average over 2 years with 7-h mean concentrations of 61 and 65 ppb O_3, compared with CF treatment with a 7-h mean concentration of 20 ppb (Mulchi et al., 1995; Rudorff et al., 1996a,c). In the above study, different amounts of O_3 were added above ambient levels for 5 days as follows: 20, 30, 40, 50, 60 ppb, except that O_3 was not added to exceed a total concentration of 120 ppb (Rudorff et al., 1996c). Wheat grown in pots in OTCs was exposed to elevated O_3 and water stress in Germany, and yield was decreased by 35% in the 2×-ambient treatment with a 7-h mean O_3 concentration of 71 ppb, statistically significant effects were not seen in the 1×-ambient treatment (Fangmeier et al., 1994). In two studies conducted in Raleigh, NC, soft red winter wheat was grown in pots and exposed to CF, NF, and 1.5×-ambient O_3, with ambient and elevated CO_2 concentrations (Table AX9-16) (Heagle et al., 2000). In the first experiment, eight cultivars were exposed to 12-h mean O_3 concentrations of 27, 47, and 90 ppb, and in the second experiment two of these cultivars were exposed to 22, 38, and 74 ppb. There was a trend toward decreased yield in both experiments, but these trends were not statistically significant. The wheat cultivar Drabant was exposed to CF, NF, and a constant addition of 35 ppb during 1992 and 1993 in Finland (Ojanpera et al., 1998), using the Heagle-type OTCs (Heagle et al., 1973). The following 12-h mean O_3 exposures were observed in 1992: 14, 30, 61 ppb. In 1993, the values were 9, 21, and 45 ppb, (see Table AX9-16 for AOT40 values and other information). Yield was reduced 13% in each year by the added O_3 treatment.

The effect of an intermittent constant addition of O_3 using a free air exposure system was investigated with the winter wheat cultivar Riband in Northumberland, UK (Ollerenshaw and Lyons, 1999). Ozone exposures expressed as AOT40 values for September and October 1992 were 0.14 and 3.5 ppm·h; while for April to August 1993, values were 3.5 and 6.2 ppm·h. Yield was reduced by 13%.

These results provide an additional line of evidence supporting the OTC-studies that demonstrated yield reductions in wheat due to O_3 exposures that occur in the United States. These yield reductions for wheat are generally similar to those reported previously in 22 comparable studies summarized in the 1996 AQCD (Table 5-25 of U.S. Environmental Protection Agency, 1996).

AX9.5.4.2 Effects on Plant Quality

In addition to reductions in biomass or crop yield, O_3 may also reduce the quality or nutritive value of annual species. Many studies have shown effects of O_3 on various measures of plant organs that affect quality, with most studies focusing on characteristics important for food or fodder (U.S. Environmental Protection Agency, 1996).

The effect of a continuous intermittent addition of O_3 using a free air exposure system in Northumberland, UK was investigated with the oilseed rape cultivar Eurol as discussed above (Ollerenshaw et al., 1999). Ozone exposures expressed as AOT40 values for August to October 1991 were 0.2 and 3.8 ppm·h; for June 1992 they were 0.7 and 8.1 ppm·h. Yield quality measured as crude protein and oil content was decreased significantly. Because the price of the product is reduced in direct proportion to the oil content, such a decrease represents a substantial loss to growers (Ollerenshaw et al., 1999).

Two wheat cultivars, Massey and Saluda, were each grown for one year each in Beltsville, MD (Table AX9-16) and exposed to either CF or an addition of 40 ppb for 7 h/day for 5 days/week (Mulchi et al., 1995; Rudorff et al., 1996a,c). Milling and baking quality scores and flour protein were not significantly affected by elevated O_3 exposure, but the softness equivalent was increased slightly (2.4%) in both experiments (Rudorff et al., 1996b). The authors concluded that these changes, along with other slight changes due to an increased CO_2 treatment, suggested that O_3 and CO_2 had only minor effects on wheat grain quality. In wheat grown in

Sweden, the harvest index was significantly decreased and the protein content increased due to exposure to a 12-h mean of 48 ppb (Gelang et al., 2000). In an analysis of 16 experiments conducted with spring wheat and either O_3 or CO_2 exposures in four Nordic countries, a negative linear relationship was found between grain yield and grain protein content ($y = -0.38\times + 138.6$, expressed as percentages of the NF treatment (Pleijel et al., 1999a).

For three soybean cultivars grown in Raleigh, NC, O_3 significantly decreased oleic acid content, although the authors stated that the reduction was not large enough to be economically important (Heagle et al., 1998b).

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

In the CHIP program the effects of O_3 were studied using OTCs at six sites in northern Europe, and yield decreases were observed as described above. The reducing sugar and starch content of tubers decreased linearly due to O_3 exposure, while the ascorbic acid concentration increased linearly (Vorne et al., 2002). Compared to the CF treatment, exposure to an AOT40 value of 14 ppm·h decreased starch concentrations by 2%, decreased reducing sugar concentration by 30%, and increased ascorbic acid concentration by 20%. While the changes in reducing sugars and ascorbic acid increase tuber quality, the reduction in starch concentration decreases tuber quality.

In two 1-year studies using OTCs in commercial fields in Spain, the soluble solids content of watermelon was decreased 4 to 8% due to seasonal O_3 exposures as follows: AOT40 = 5.96 ppm·h and SUM06 = 0.295 ppm·h in one year; and AOT40 = 18.9, SUM06 = 4.95 in the second year (Gimeno et al., 1999).

AX9.5.4.3 Effects on Foliar Symptoms

For most annual crop species, the most important effects of O_3 are on yield of the commercially important part of the crop, expressed as the mass of the harvested portion. However, for some crops, foliar symptoms are important if they reduce the marketability of the crop. This is why efforts have been made to identify O_3 exposures associated with foliar symptoms. In Europe, Level I critical levels have been determined for such effects based on observations from experiments conducted in 15 countries under the auspices of the United Nations Economic Commission for Europe International Cooperative Programme on effects of air pollution and other stresses on crops and non-woody plants (UN ECE ICP-Vegetation; formerly ICP-Crops), as well as on observations of symptoms in commercial fields from 1993 to 1996 (Benton et al., 1995; Benton et al., 2000). Because the occurrence of symptoms increased with greater humidity, these levels took into account the VPD. Two short-term critical levels were derived from 1995 data: an AOT40 value of 0.2 ppm·h over 5 days when mean VPD is below 1.5 KPa (0930 - 1630 h), and a value of 0.5 ppm·h when the mean VPD is above 1.5 Kpa (Benton et al., 1996). The 1996 data supported the critical levels in 83% of observations, although symptoms occurred on three occasions when the AOT40 was less than 0.05 ppm·h and the VPD was very low — less than 0.6 Kpa. The authors concluded that these critical levels are good indicators of the likelihood of foliar symptoms, but that further refinement may be required, such as including factors that modify O_3 uptake by stomata.

In a more recent study in Germany, 25 native herbaceous species were exposed to several square-wave O_3 exposures in CF OTCs (Bergmann et al., 1999). Six of the 25 species showed O_3-specific symptoms, and five species responded to single-day peaks. The most sensitive species exhibiting O_3-specific symptoms were *Cirsium arvense* and *Sonchus asper*, both of which responded to AOT40 values < 1.5 ppm·h (Bergmann et al., 1999).

AX9.5.4.4 Other Effects

Several studies during recent decades have demonstrated O_3 effects on different stages of reproduction. Effects of O_3 have been observed on pollen germination, pollen tube growth, fertilization, and abortion of reproductive structures, as reviewed by Black et al. (2000). This issue is not addressed here, because reproductive effects will culminate for seed-bearing plants in seed production, and the substantial body of evidence relating O_3 exposure and reduced seed

production was discussed above. However, one example of a native species will be presented, because of its implications for extrapolating exposure-response data to noncommercial species. Spreading dogbane (*Apocynum androsaemifolium* L.) has been identified as a useful species for O_3 biomonitoring, because of O_3-induced diagnostic symptoms (Kohut et al., 2000). A study in Massachusetts found that exposure to O_3 in NF OTCs or ambient plots for 103 days produced significantly fewer flowers and that fewer of these flowers survived to produce mature fruits (Bergweiler and Manning, 1999). Because foliar symptoms were not common, the authors concluded they are not required for effects on reproduction to occur. Genotoxic effects and effects on population genetics were discussed in Section AX9.3.

AX9.5.4.5 Scaling Experimental Data to Field Conditions

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

While it is clear that chambers can alter some aspects of plant growth (for example, (Bytnerowicz et al., 2004; Elagöz and Manning, 2005b), the more important issue is whether they alter the response of plants to O_3. A review of such chamber studies done in California found that plants responded similarly to O_3 whether OTCs, closed-top chambers, or air exclusion systems were used; differences were found for fewer than 10% of growth parameters (Olszyk et al., 1986a). In another review of literature about Heagle-type OTCs (Heagle et al., 1988), the authors concluded that "Although chamber effects on yield are common, there are no results showing that this will result in a changed yield response to O_3." A more recent study of chamber effects examined the responses of tolerant and sensitive white clover clones to ambient O_3 in greenhouse, OTCs, and ambient plots (Heagle et al., 1996). For individual harvests, O_3 reduced the forage weight of the sensitive clone 7 to 23% more in the greenhouse than in OTCs. However, the response in OTCs was the same as in ambient plots. A similar study with these

white clover clones near Naples, Italy also found no significant difference between O_3 effects measured in OTCs versus those measured by comparing the ratio of sensitive and resistant clones in ambient air (Fagnano et al., 2004). Several studies have shown very similar yield responses to O_3 for plants grown in pots or in the ground, suggesting that even such a significant change in environment does not alter the proportional response to O_3, at least as long as the plants are well- watered (Heagle, 1979; Heagle et al., 1983). As discussed in Section AX9.1, results from recent FACE studies are similar to those obtained from OTC studies, providing another line of evidence that chamber effects in OTCs do not substantially alter O_3 exposure-response relationships (Morgan et al., in press).

Most experiments investigating O_3 effects on annual vegetation provide adequate water to avoid substantive drought stress. Because drought stress has generally been shown to reduce the effect of O_3 on annual vegetation, such experiments may tend to overestimate O_3 effects on crops and especially on unmanaged or seminatural vegetation.

As mentioned above, the use of O_3 flux, rather than exposure, is theoretically more realistic, and such an approach would also address the vertical gradient issue (Section 3.3.2, Section AX9.1). A number of investigators have suggested that modeling O_3 flux can improve estimates of O_3 effects on vegetation. Models of O_3 flux can reduce the variation in the response to O_3 that is sometimes observed between years in an experiment (Emberson et al., 2000a, b; Fuhrer et al., 1992; Grünhage et al., 1993; Grünhage and Haenel, 1997; Pleijel et al., 2000a). In a study of O_3 deposition to an oat crop in OTCs, O_3 flux in the chamber was estimated to be up to twice that in an adjacent field based on a K-theory approach and measurements of stomatal conductance and environmental conditions (Pleijel et al., 1994b). These measurements were made for 2 hours on 5 days when the canopy was physiologically active and wind speeds were moderate. However, the O_3 flux in a plant-less chamber was nearly as high as that in the open field. The authors conclude that O_3 uptake in the chamber was between 100 and 200% of that in the field. These models of flux have a sound biological and meteorological basis and are useful for interpreting experimental data. Flux models have been successfully applied at intensive study sites with detailed site-specific data on stomatal conductance and micrometeorological conditions (e.g., Fredericksen et al., 1996b; Grünhage et al., 1993, 1994)). Yet even at a single well-studied site, different methods can provide different estimates of O_3 flux. For example, at a site in a vineyard in California, an evapotranspiration-based method overestimated the O_3 flux as

compared to an eddy covariance approach by 20 to 26% (Massman and Grantz, 1995). At a site in a nearby cotton field the evapotranspiration-based approach overestimated the eddy-covariance method by 8 to 38%. Flux-response relationships have received substantial attention in Europe during the past decade, as part of an attempt to move beyond the exposure-based Level 1 critical levels to flux-based Level II critical levels (Section AX9.4). However, the database for flux-response relationships is very limited (Grünhage et al., 2004), and these approaches do not always explain greater amounts of response variation than do exposure-based approaches (Karlsson et al., 2004b). The critical level approach is discussed briefly below, because it has been used to extrapolate from field studies to landscapes, countries, and regions.

There has been criticism that the Level I critical level for crops overestimates O_3 effects in the Mediterranean countries, because it was developed based on studies in Northern Europe (De Santis, 1999; De Santis, 2000). However, there is evidence of substantial crop loss due to O_3 in some southern European counties, such as the Po valley in Northern Italy (Fumagalli et al., 2001). In these studies, Heagle-type OTCs were used. Losses in NF chambers as compared to CF chambers over several years at two sites ranged from 11.2 to 22.8% for barley and wheat, from 0.3 to 31.5% for other crop species, and from 4.1 to 19.8% for forage species (Fumagalli et al., 2001). Surprisingly, the least effect was observed for soybean, despite AOT40 values of 9.32 ppm·h, 3× the Level I critical level. Similarly, a review of studies in Northern Italy found that ambient O_3 episodes have been reported to cause foliar symptoms on 24 agricultural and horticultural crops in commercial fields (Fumagalli et al., 2001). Ambient O_3 has also been reported to cause yield losses in several crop species, although no data on O_3 exposure were presented by the reviewers (Fumagalli et al., 2001).

The Level I approach has also been criticized for focusing only on a single annual species (wheat) and a single woody perennial species (beech). However, this species focus is appropriate, because the goal was to determine an exposure-response relationship for a sensitive species based on available data. In support of standards in Germany, an effort was made to combine data from different species, and consisted of a meta-analysis of studies conducted in both closed and OTCs (Grünhage et al., 2001). In this study, experiments published between 1989 and 1999 were included and analyzed if they met the following conditions: (1) a significant O_3 effect was determined; (2) exposure conditions were well defined; (3) foliar symptoms, growth, or yield was measured; and (4) plant species were relevant to Europe

(Grünhage et al., 2001). Despite the focus on European species, many of the species studies also occur in the United States. Separate regressions for herbaceous plants and for tree species were created as a function of duration of exposure at a given level of O_3 exposure at the top of the plant canopy. These regression equations, with confidence limits and with correction for the vertical gradient in O_3 from the top of the quasi-laminar boundary layer, can be used to define whether effects are unlikely (below the lower confidence limit), probable (between the confidence limits), or highly likely (above the upper confidence limit) to occur near a given O_3-monitoring station.

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

Exposure-response relationships developed primarily from OTC experiments, with confirming evidence from other approaches such as resistant and sensitive clover clones exposed in ambient air and FACE experiments, are useful for estimating the effects of ambient O_3 on vegetation in the U.S. However, despite the substantial number of experimental studies listed in Table AX9-16 and in previous Air Quality Criteria Documents for O_3 (for example, U.S. Environmental Protection Agency, 1996), most studies have been conducted in only a few locations with only a few species of economically important crops. While these studies provide strong evidence that ambient O_3 in the U.S. is likely reducing crop yields and plant growth significantly in many regions in many years, it remains difficult to extrapolate to all landscapes and all plant species and to determine whether exposure-response relationships based on existing studies will protect all species in all locations from significant deleterious effects of O_3.

AX9.5.4.6 Summary of Effects on Short-Lived Species

For annual vegetation, the data summarized in Table AX9-16 show a range of growth and yield responses both within species and among species. Nearly all of these data were derived from studies in OTCs, with only two studies using open-air systems in the UK (Ollerenshaw et al., 1999; Ollerenshaw and Lyons, 1999). It is difficult to compare studies that report O_3 exposure in different indices such as AOT40, SUM06, or 7-h or 12-h mean values. However,

when such comparisons can be made, the results of this more recent body of research confirm the earlier results summarized in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). A summary of earlier literature concluded that a 7-h, 3-month mean of 49 ppb corresponding to a SUM06 exposure of 24.4 ppm·h would cause 10% loss in 50% of 49 experimental cases (Tingey et al., 1991). A similar study using a 24-h, rather than 7-h, averaging period found that a SUM06 exposure of 26.4 ppb would cause 10% loss in 50% of 54 experimental cases (Lee et al., 1994a,b). Recent data summarized in Table AX9-16 support this conclusion, including data from a FACE study which confirmed results found previously using OTCs (Morgan et al., in press). These values represent ambient exposure patterns that occur in some years over large portions of the United States. Some annual species such as soybean are more sensitive, and greater losses may be expected (Table AX9-16). Thus, the recent scientific literature supports the conclusions of the 1996 AQCD that ambient O_3 concentrations are reducing the yield of major crops in the United States.

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

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

The use of OTCs may reverse the usual vertical gradient in O_3 that occurs within a few meters above the ground surface (Section 3.3.2, Section AX9.1). Such a reversal suggests that

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

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

Although there has been considerable research in Europe on annual species during the past 10 years, much research in the United States has focused on perennial species. In Europe, and in a few studies in the United States, effects of O_3 on mixtures of annual and perennial herbaceous species have been investigated using growth chambers, greenhouses, and OTCs. Section AX9.5.5.1 reviews such studies, with an emphasis on studies using OTCs.

AX9.5.5.1 Herbaceous Perennial Species

Two alfalfa cultivars were grown in pots and exposed to CF, NF, 1.5×-ambient and 2×-ambient O_3 concentrations in two 1-year studies in Quebec, Canada (Renaud et al., 1997). One cultivar, Apica, is commonly grown in the region, and another, Team, is normally grown farther south and is more tolerant to O_3. For Apica in both years and for Team in 1991, O_3 exposure caused a linear reduction in above-ground biomass. In the NF treatment, growth of Apica was decreased by 31 and 21% in the 2 years, while the growth of Team was reduced by 14% in 1991, but not reduced in 1992. The authors suggested that the differing effects on Team could be due to different progenies and propagation methods in the 2 years or to more rapid growth in 1991 along with higher O_3 peak values in 1991. In 1991, O_3 maxima exceeded 60 ppb in 15 days, whereas in 1992 there were only three such days. At the end of the growing season,

total starch reserves in roots were decreased by O_3, due primarily to a decrease in root mass, that the authors suggested could accelerate decline in alfalfa yields. These yield reductions are generally similar to those reported previously in five similar studies summarized in the 1996 AQCD (Table 9-25 of U.S. Environmental Protection Agency, 1996).

A study in Alabama exposed early- and late-season-planted Bahia grass (*Paspalum notatum* cultivar Pensacola) in OTCs to CF, NF, or 2×-ambient O_3 treatments (Muntifering et al., 2000). Ozone exposures expressed as 12-h mean values over the 24-week experiment were 22, 45, and 91 ppb, and the highest ambient O_3 concentrations were recorded in late June, late July, late August and mid-September at approximately 90 ppb. Above-ground biomass growth was reduced by the NF treatment for the first and second harvest by 34% and 29% for the early-season planting, but statistically significant effects were not observed in the late-season planting (Table AX9-17). The 2×-ambient treatment did not cause further significant reductions in biomass. The authors suggested that the lack of a significant O_3 effect in the late planting may have been due to the shorter total O_3 exposure time as well as to the lower O_3-exposure concentrations during the weeks immediately preceding harvest. These results are important, because this is an economically important species and because previous studies have focused on grass species that use the C_3, rather than C_4, metabolic pathway.

An investigation of the use of different O_3 indices and averaging times on the correlation with growth effects was undertaken with the North Carolina clover system (Heagle et al., 1995). For 2 years of data at six sites in Massachusetts, Oregon, North Carolina, California (2 sites), and Virginia, averaging time was found to be more important than the choice of the type of index including mean, SUM06, and AOT40 (Heagle and Stefanski, 2000). The best correlation between O_3 exposure and the ratio of sensitive-to-tolerant clover types was found for the 6-h period from 1000 to 1600 h. For this period, very similar r^2 values (0.91 to 0.94) were found for SUM06, W126, and AOT40 (Heagle and Stefanski, 2000). For the above indices, a linear relationship was found, with no effect in Corvallis, OR with exposure to a SUM06 value of 10.2 ppm·h and a ratio of 0.53 (sensitive/tolerant) at San Bernardino with a SUM06 exposure of 39.4 ppm·h (Heagle and Stefanski, 2000).

In a study of the biomass ratio of O_3-sensitive versus O_3-insensitive clover at 14 sites in Europe during 1996 to 1998, a model was developed using ANN techniques (see Section AX9.1) that had r^2 values for the training data of 0.84 and for unseen validation data of 0.71 (Mills et al.,

2000). The predictive factors in the model were AOT40, 24-h mean O_3, daylight mean temperature, and 24-h mean temperature. This model was selected after a thorough investigation of a number of models using many more or fewer parameters using both ANN and multiple linear regression techniques. This model predicted that a 5% reduction in biomass ratio was associated with AOT40 values in the range 0.9 to 1.7 ppm·h accumulated over 28 days, with plants being most sensitive under conditions of low NO_x, moderate temperature, and high 24-h mean O_3 concentration.

Exposure to a square-wave 8-h mean O_3 concentration of 92 ppb for 62 days in an experiment in OTCs the UK did not significantly reduce the total yield of strawberry fruits, but did decrease the average size of the fruits by 14% (Drogoudi and Ashmore, 2000). This contrasts with an increase in total yield (fruit weight) found in a previous study in California (Takemoto et al., 1988).

When timothy was exposed in OTCs in Sweden to NF, CF, and CF+O_3 treatments, there was no effect of a 12-h mean O_3 exposure of 68 ppb (NF treatment), but a 12-h mean exposure of 152 ppb decreased yield by 58% (Danielsson et al., 1999). A similar lack of effect of exposure to a 12-h mean O_3 exposure of 62 ppb was found in a previous study in the United States (Kohut et al., 1988).

Although most investigations of O_3-response relationships focus on growth or yield of marketable portions of plants, some studies also investigate effects on plant quality. In the study of bahia grass in Alabama discussed above, in addition to the effects on yield, there were significant effects on quality for ruminant nutrition (Muntifering et al., 2000). Concentrations of neutral detergent fiber (NDF) were higher in primary-growth and regrowth forages from the early-season planting when exposed to 2×-ambient O_3 than when exposed to the NF treatment. The concentration of acid detergent fiber was higher in the 2×-ambient treatment than in NF treatment regrowth, whereas acid detergent lignin concentration was higher in 2×-ambient than in NF primary-growth forage. Crude protein concentrations were lower in CF-exposed than in NF-exposed regrowth forage from the early planting and in CF- than in NF-exposed primary-growth forage from the initial harvest of the late-season planting. No differences were observed among treatments in concentrations of total phenolics in primary-growth or regrowth forages from either planting, although concentrations of total phenolics tended to be higher in CF-exposed than in NF-exposed primary-growth forage from the late-season planting. The

authors concluded that the alterations in quality of primary-growth and vegetative regrowth forages were of sufficient magnitude to have nutritional and possibly economic implications to their use for ruminant animal feed.

Sericea lespedeza and little bluestem were exposed to CF, NF, and 2×-ambient O_3 in OTCs in Alabama for 10 weeks (Powell et al., 2003). Ozone treatments expressed as 12-h mean concentrations were 23, 40, and 83 ppb and expressed as seasonal SUM06 values were 0.2, 9.1, and 61.0 ppm·h. Although there were few statistically significant effects of O_3 on yield (the yield of only the 2×-ambient compared to NF for *Sericea lespedeza* in the last of six harvests), plant quality as feed for ruminants was reduced. The nutritive quality of *Sericea lespedeza* was decreased by 7% and that of little bluestem by 2% as a result of increased cell wall constituents and decreased in vitro digestibility.

For some annual species, particularly crops, the endpoint for an assessment of the risk of O_3 exposure can be defined as yield or growth; e.g., production of grain. For plants grown in mixtures such as hayfields, and natural or seminatural grasslands (including native nonagricultural species), endpoints other than production of biomass may be important. Such endpoints include biodiversity or species composition and measures of plant quality such as total protein and effects may result from competitive interactions among plants in mixed-species communities. Most of the available data on non-crop herbaceous species are for grasslands.

In a study of two perennial grasses (bent grass and red fescue) and two forbs (white clover, Germander speedwell [*Veronica chamaedrys* L.]) grown in pots in OTCs, O_3 effects differed among species and cutting treatments (Ashmore and Ainsworth, 1995) (see also Table AX9-17). Fescue biomass increased with higher O_3 treatments both in pots that were not cut during the growing season (mid-June to mid-September) and those that were cut every two weeks. However, bent grass biomass decreased with higher O_3 exposure in the uncut treatment and increased in the cut treatment. White clover and Germander speedwell biomass decreased substantially with higher O_3 exposure with and without cutting, with greater decreases in the cut treatment. The authors cautioned that the experiment did not replicate field circumstances. The plants were all cut to 1 cm above the ground, which does not simulate grazing, and there may have been effects due to growing the plants in pots. However, two key results of this study likely apply to mixtures of species growing in hay or forage fields or seminatural and natural communities. First, O_3 exposure increased the growth of O_3-tolerant species, while exacerbating

the growth decrease of O_3 sensitive species. Second, the total biomass of the mixed-species community was unaffected by O_3 exposure due to the differential effects on O_3-sensitive and O_3-tolerant species.

In a 2-year study using OTCs placed over managed pasture in Switzerland, the above-ground biomass of clover (red and white) was reduced linearly in response to increased O_3 exposure (Table AX9-17) (Fuhrer et al., 1994). Exposure to a 12-h mean concentration of 39 ppb O_3 reduced biomass by 24% as compared to the CF treatment with a 12-h mean concentration of 21 ppb O_3. There was a trend toward increased above-ground biomass of grasses (primarily orchard grass), but this trend was not statistically significant. As often found in other studies of mixtures of species, by O_3 exposure did not significantly affect total above-ground biomass O_3 exposure.

A field-grown grass/clover mixture was exposed to CF, NF, and two O_3 addition treatments for two growing seasons in OTCs in southern Sweden (Pleijel et al., 1996). The mixture consisted of 15% (by seed weight) red clover cv. Fanny, 60% timothy cv. Alexander, and 25% fescue cv. Svalofs Sena. Ozone concentrations expressed as AOT40 ranged from 0 to approximately 47 ppm·h and expressed as 7-h mean from 11 to 62 ppb. Over this range, a slight, but statistically significant, linear decrease of 4% in total above-ground biomass was seen growth over six harvests. No significant decrease was seen in the proportion of clover, and the authors ascribed this lack of effect to the relatively higher O_3 sensitivity of timothy and lower sensitivity of this clover cultivar as compared to previously published results for other grass/clover mixtures (e.g., Fuhrer et al., 1994).

A mixture of species in an old farm field in Alabama was exposed to O_3 for two growing seasons in large OTCs (4.8 m high and 4.5 m diameter); and a similar lack of effect of O_3 was found on total plant community growth measured as both canopy cover and vertical canopy density (Barbo et al., 1998). Of the 40 species in the plots, O_3 effects were examined only on the most common species: blackberry, broomsedge bluestem, bahia grass, *Panicum* spp., and winged sumac (second year only). Of these species, a 2×-ambient O_3 treatment increased the percent canopy cover of blackberry over 2 years by 124%, while that of winged sumac was decreased by 95% (Table AX9-17). Blackberry showed no significant effect on biomass, but ripe fruit mass was decreased by 28% (Chappelka, 2002). However, there was a significant chamber effect for this latter response. Biomass was not reported for other species in this study

due to a hurricane. Effects on loblolly pine grown in this experiment are discussed subsequently in Section AX9.5.5.5.

In summary, results of studies on perennial herbaceous species conducted since the 1996 criteria document was prepared are presented in Table AX9-17. As for single-season agricultural crops, yields of multiple-year forage crops are reduced at O_3 exposures that occur in some years over large areas of the United States (Tables AX9-13, AX9-21). This result confirms that reported in the 1996 AQCD (U.S. Environmental Protection Agency, 1996). When species are grown in mixtures, O_3 exposure can increase the growth of O_3-tolerant species while exacerbating the growth decrease of O_3-sensitive species (e.g., Ashmore and Ainsworth, 1995; Fuhrer et al., 1994). Because of this competitive interaction, the total growth of the mixed-species community may not be affected by O_3 exposure (Ashmore and Ainsworth, 1995; Barbo et al., 1998; Fuhrer et al., 1994). However, in some cases mixtures of grasses and clover species have shown significant decreases in total biomass growth in response to O_3 exposure in studies in the United States (Heagle et al., 1989; Kohut et al., 1988) and in Sweden (Pleijel et al., 1996). In Europe, a provisional critical level for perennials of an AOT40 value of 7 ppm·h over 6 months has been proposed to protect sensitive plant species from the adverse effects of O_3.

AX9.5.5.2 Deciduous Woody Species

It is extremely difficult and costly to study entire mature trees under controlled conditions such as those in OTCs, with the possible exception of some species managed for fruit or nut production. For this reason, the great majority of investigations have been of seedlings in growth chambers, greenhouses, or OTCs. A few investigations have been carried out on saplings or more mature trees using free air exposure systems (Haeberle et al., 1999; Isebrands et al., 2000, 2001; Werner and Fabian, 2002). Exposure-response functions based on 28 experimental cases of seedling response to O_3 suggest that a SUM06 exposure for 3 months of 31.5 ppm·h would protect hardwoods from a 10% growth loss in 50% of the cases (Table AX9-18). However, there is a substantial range in sensitivity among species. A risk analysis was undertaken to predict tree biomass growth reductions due to O_3 based on exposure-response equations for seedlings of individual species combined with the species' spatial distribution across the eastern United States and interpolated O_3 exposure expressed as SUM06 (Hogsett et al., 1997). The growth of sensitive species such as aspen and black cherry was predicted to be

reduced by at least 20% across 50% of their ranges in a high O_3 year and approximately 10% in a lower-than-average O_3 year (Hogsett et al., 1997).

A few investigations reported since the last criteria document was prepared have examined saplings or mature trees, notably of oak species in the southern Appalachian Mountains and pine species in California. Most of these studies have been of natural (uncontrolled) O_3 exposures. Additional studies have examined foliar symptoms on mature trees, and in recent years such surveys have become more common and with greater attention to the standardization of methods and the use of reliable indicator species (Campbell et al., 2000; Smith et al., 2003). Previous criteria documents have noted the difficulty in relating foliar symptoms to effects on individual tree growth, stand growth, or ecosystem characteristics (U.S. Environmental Protection Agency, 1996). This difficulty still remains to the present day.

Some investigators have suggested that a comprehensive risk assessment of the effects of O_3 on mature tree species might best be accomplished by extrapolating measured effects of O_3 on seedlings to effects on forests using models based on tree physiology and forest stand dynamics (Chappelka and Samuelson, 1998; Laurence et al., 2000, 2001). Several such efforts are discussed in Sections AX9.3 and AX9.6.

In this subsection, emphasis will be placed on experimental evidence of O_3 effects on the growth of woody species under controlled conditions with some information from observational studies under ambient conditions in forests. Experimental results are summarized for deciduous species in Table AX9-18; the species are discussed below in the order in which they appear in this table.

A series of studies in Michigan and Wisconsin during the 1990s on clones of trembling aspen previously demonstrated that they differ in their O_3 sensitivity (Coleman et al., 1995a,b, 1996; Dickson et al., 2001; Isebrands et al., 2000, 2001; Karnosky et al., 1996, 1998, 1999; King et al., 2001). Several of those studies were undertaken with plants in pots or in the ground in OTCs and additional studies were undertaken at three sites selected to differ primarily in O_3 exposure (Karnosky et al., 1999). An ongoing study was undertaken using a FACE carbon dioxide and O_3 enrichment facility in Rhinelander, WI (Isebrands et al., 2000, 2001). These studies showed that O_3-symptom expression was generally similar in OTCs, FACE, and gradient sites, supporting the previously observed variation among aspen clones (Karnosky et al., 1999). In the Michigan OTC study, plants were grown in pots and exposed to CF, 0.5× ambient, 1×

ambient, 1.5× ambient, 2×-ambient O_3 treatments for 98 days (Karnosky et al., 1996). Ozone concentrations expressed as 3-month, 7-h mean values were 7 ppb (CF), 43 ppb (1×) and 63 ppb (2×). Ozone decreased total plant biomass between 2 and 22% for three clones previously selected to represent high, intermediate and low O_3 tolerance based on previous studies of larger populations(clones 216, 271 and 259; Karnosky et al., 1996). Seedlings produced from 15 parent trees responded similarly as did the 3 clones, with an average biomass reduction of 14% in the 1×-ambient treatment for the seedlings compared to 16% for the clones. In a second experiment using square wave exposures, biomass reduction for the clones was ranged from 23 to 39% (mean = 31%) at a 7-h mean O_3 concentration of only 45 ppb, which is similar to the response to the 2×-ambient treatment in the previous experiment at a 7-h mean O_3 concentration of 66 ppb.

The FACE study evaluated the effects of multiple years of exposure to combinations of elevated CO_2 and 1.5× ambient O_3 on growth responses in mixture of five trembling aspen clones (Isebrands et al., 2000, 2001). Height, diameter, and stem volume (diameter^2 × height) were decreased by elevated O_3. On average for all clones, stem volume was decreased by 20% over the first 3 years in the elevated O_3 treatment as compared with the 1×-ambient treatment. However, one clone showed increased growth in response to O_3. Ozone concentrations were not reported. Over the first 7 years of the study, average stem volume was decreased by 14% with 12-h mean O_3 concentrations between 49 and 55 ppb as compared with effects at ambient O_3 concentrations with 12-h mean values of 35 to 37 ppb (O_3 exposure data are for the first 4 years, as they have not been reported for subsequent years) (Karnosky et al., 2003b, 2005). This FACE facility study is important, because it confirmed responses reported previously with these clones grown in pots or soil in OTCs, without the alterations of microclimate induced by chambers. Currently, this is the only U.S. study using this technology to have examined the effects of O_3 under these conditions. This study is also significant, because the elevated O_3-exposure pattern used was intended to reproduce the 6-year average pattern from Washtenaw County, Michigan (Karnosky et al., 1999).

Rooted cuttings of two aspen clones from Acadia National Park in Maine were exposed to 1×-ambient, 1.7×-ambient, and 3×-ambient O_3 concentrations in large OTCs in Ithaca, NY for much of one growing season (15 June to 15 September) (Yun and Laurence, 1999a). Both circular (4.7 m diameter, 3.7 m height) and rectangular (7.4 m × 2.75 m × 3.7 m height)

chambers were used (Mandl et al., 1989). Exposure to 1.7×-ambient O_3 (SUM06 = 20 ppm·h, 9-h mean = 74 ppb) reduced shoot growth by 14 and 25% compared to ambient O_3 for the two clones (Yun and Laurence, 1999a). Total dry weight was reduced by 55 and 35% in the two clones by the 3×-ambient treatment (SUM06 = 62 ppm·h, 9-h mean = 124 ppb) compared to the ambient O_3 treatment.

When black poplar cuttings in OTCs in Belgium were exposed to 8-h mean O_3 concentrations of 5, 29, and 33 ppb, diameter growth decreased by 29% in the highest O_3 treatment, but height growth was unaffected (Bortier et al., 2000b). A 2-month study of hybrid poplar (*Populus tremuloides* × *P. tremul*a) in a free air exposure system in Finland with 7-h mean O_3 concentrations of 30 and 38 ppb found a 6% decrease in height with no effect on biomass (Oksanen et al., 2001). Eastern cottonwood cuttings in pots buried in the ground with drip irrigation were exposed to ambient O_3 at several sites in and near New York City in three 2-month experiments during three summers (Gregg et al., 2003). Ozone concentrations were lower at urban sites than at rural sites within 100 km of the urban sites. Total biomass growth was greater in urban than rural sites, with a strong linear decrease in biomass with increasing O_3 across all sites and years (r^2 = 93). Total biomass decreased 33% with 12-h mean O_3 levels of 38 ppb compared to 23 ppb. Multiple regression analysis showed no significant temperature effect on biomass. Therefore, the authors suggested that O_3 exposures were the most likely explanation for the reduced biomass in rural areas. The overall growth reductions and the variation among genotypes seen on all of the above aspen and poplar studies is similar to those previously reported in three OTCs studies summarized in the 1996 O_3 AQCD (Table 9-26 of U.S. Environmental Protection Agency, 1996).

For paper birch, over the first 7 years of the Wisconsin FACE study, average stem volume was unaffected by 12-h mean O_3 concentrations between 49 and 55 ppb as compared to effects at ambient O_3 concentrations with 12-h mean values of 35 to 37 ppb (based on O_3 exposure data for the first 4 years, as they have not been reported for subsequent years) (Karnosky et al., 2003b, 2005). In contrast, significant effects were found in this study for aspen and sugar maple, so these results indicate that paper birch is relatively insensitive to O_3 compared to these other species.

Black cherry seedlings grown in pots were exposed in OTCs in the Great Smoky Mountain National Park in Tennessee to O_3 treatments ranging from CF to 2×-ambient in two experiments

during 1989 and 1992 (Neufeld et al., 1995). Ozone exposure, expressed as SUM06, ranged from 0 to 40.6 ppm·h in 1989 and from 0 to 53.7 ppm·h in 1992. Corresponding AOT40 values were 0.0 to 28.3 ppm·h in 1989 and 0 to 40.4 ppm·h in 1992. In 1989, total biomass was decreased in the 1.5×-ambient treatment by 18% and in the 2×-ambient treatment by 38%. In 1992, total biomass was decreased in the 1.5×-ambient treatment by 27%, and in the 2×-ambient treatment by 59%. In this study, SUM06 and AOT40 provided better fits than did SUM00 with Weibull regression analyses to log-transformed biomass data. Although a Weibull model was used, responses to O_3 expressed as SUM06 and AOT40 appeared to be linear. The O_3 exposures in the 1.5×-ambient and 2×-ambient treatments were reported to be similar to those for a site near Charlotte, NC in a high-O_3 year (1988). In a 2-year experiment in OTCs in Ohio, seedlings of black cherry, sugar maple, and yellow poplar were exposed to O_3 treatments with SUM00 values ranging from 16 to 107 ppm·h in 1990 and 31 to 197 ppm·h in 1991 (Rebbeck, 1996). After two seasons of exposure, only black cherry showed a growth decrease: total biomass was reduced by 32% in the 2×-ambient O_3 treatment compared to the CF treatment; root biomass was decreased by 39%. These results contrast with those of a previous study with black cherry seedlings in which significant biomass reductions with exposures up to 2×-ambient were not observed (7-h mean = 21 to 97 ppb), perhaps because of the small sample size (3 seedlings per chamber (Samuelson, 1994) in the earlier study.

A multiyear study of effects of O_3 on both seedling and mature (30-year-old) red oak trees was conducted in Norris, TN in large OTCs with three replicates per O_3 treatment. Trees were exposed for 3 years to CF, 1×-ambient, and 2×-ambient treatments, with the following O_3 exposures: SUM06 for 3 years = 0, 29, 326 ppm·h; SUM00 for 3 years = 147, 255, and 507 ppm·h. The net photosynthetic rate in mature trees was reduced by 25% in the ambient treatment and by 50% in the 2×-ambient treatment (Samuelson and Edwards, 1993; Hanson et al., 1994; Wullschleger et al., 1996). Despite these large decreases, no significant effects on stem increment at the base, stem increment in the canopy, or leaf mass were observed for the mature trees (Samuelson et al., 1996). Similarly, seedling biomass was not significantly reduced by O_3 exposure. The difficulty in replicating experiments with mature trees makes it difficult to detect changes in growth or biomass. However, the mean values of the stem increment at the base and within the canopy in the ambient treatment were larger than those in the CF treatment, although those in the 2×-ambient treatment were lower. Therefore, this study of mature trees

does not provide evidence that these ambient concentrations reduced above-ground tree growth, even after 4-years exposure.

Sugar maple seedlings were exposed for 3 years to ambient, 1.7×-ambient, and 3×-ambient O_3 treatments at both high-light (35% of ambient) and low-light levels (15% of ambient) (Topa et al., 2001). This experiment was conducted in large OTCs near Ithaca, NY. Over the 3 years, O_3 exposures expressed as SUM00 for the three treatments were 88, 126, and 185 ppm·h. After 3 years, total seedling biomass in the 3×-ambient treatment was reduced by 64% and 41% in the low- and high-light treatments, respectively (compared to the 1×-ambient treatment). The larger reduction of biomass under low-light conditions suggests that seedlings growing under closed canopies may be substantially more sensitive to O_3 than are seedlings exposed to higher-light levels in gaps or clearings. These results differ from other studies in which seedling biomass was unaffected by exposure to SUM00 values of 304 ppm·h over 2 years (Rebbeck, 1996) or 591 ppm·h over 3 years (daytime mean of 40.7) (Laurence et al., 1996). However the latter two studies used much higher light levels, which may have reduced the O_3 effect, based on the results of Topa et al. (2001). Over the first 7 years of the Wisconsin FACE study, average stem volume of sugar maple was decreased by 14% with 12-h mean O_3 concentrations between 49 and 55 ppb as compared with effects at ambient O_3 concentrations with 12-h mean values of 35 to 37 ppb (based on O_3 exposure data for the first 4 years, as they have not been reported for subsequent years) (Karnosky et al., 2003b, 2005). These growth effects were not statistically significant for the first 3 years, but became significant subsequently. These results are important because they demonstrate that 3 years of exposure may not be long enough to evaluate effects of O_3 on the growth of tree species.

Although most studies demonstrate that O_3 decreases biomass growth, occasional results indicate that O_3 can increase growth of some portions of woody perennials. When Casselman plum trees near Fresno, CA were exposed to O_3 in large, rectangular OTCs to three O_3 treatments (CF, 1×-ambient, and an above-ambient O_3 treatment) for 4 years (12-h mean = 31, 48, 91 ppm·h), stem increment increased 14% in the highest O_3 treatment compared to the CF treatment; and this effect was statistically significant (Retzlaff et al., 1997). However, fruit yield decreased in this treatment by 42% and also decreased by 16% in the 1×-ambient-O_3 treatment. Root growth was not measured in this study. Hence, the increase in stem diameter may have been at the expense of other organs. However, in a fifth year, all plants were exposed to

1×-ambient O_3, and there were no differences in fruit yield, suggesting that trees were able to recover to some extent from the effects of O_3 exposure in prior years.

When potted yellow poplar seedlings were exposed to O_3 concentrations up to SUM00 values of 107 ppm·h in one year and 197 ppm·h in a second year, no effects on biomass were observed (Rebbeck, 1996). In a study at the same location with seedlings planted in the ground and exposed to O_3 concentrations with SUM06 values of 0.3, 228.7, and 661.8 ppm·h over 5 years, no effects on biomass were found (Rebbeck and Scherzer, 2002).

Many studies have demonstrated that root growth is more sensitive to O_3 exposure than is stem growth. For example, in a study with black cherry seedlings exposed in OTCs in Tennessee in 1989, root biomass in a 2×-ambient treatment was decreased by 42%, while stem biomass was decreased by only 24%. However, in a second experiment in 1992, root and stem growth reductions in the 2×-ambient treatment were similar (65% versus 60%) (Neufeld et al., 1995). In Finland, reduced root growth was found for a number of clones of silver birch (Oksanen and Saleem, 1999). After 5 years, root growth was decreased by 33%, but shoot growth was not affected by O_3 exposures of a 7-h mean of 15 ppm·h over 5 years in a FACE system (Oksanen, 2001). When first-year poplar seedlings (*P. tremuloides*) were exposed in OTCs to two O_3 concentrations and six N concentrations, the root/shoot ratio was decreased soon after exposure to O_3 in all N treatments, even though O_3 effects on total biomass were not detected in the low-N and very high-N treatments (Pell et al., 1995). These results suggest that effects on the root/shoot ratio occur before significant growth effects arise. In a series of OTC experiments lasting 1 to 3 years at 3 different elevations in Switzerland, fine root growth in European beech was found to be more sensitive to O_3 than was shoot or total biomass (Braun and Fluckiger, 1995). Although the estimated effect of O_3 on fine root biomass was similar to that for total biomass, fine root biomass was significantly decreased at AOT40 (24-h) values of only 3 ppm·h, while total biomass was not significantly decreased until AOT40 values reached 30 to 40 ppm·h.

AX9.5.5.3 European Critical Levels

In Europe, a Level I critical level has been set for forest trees based on OTC studies of saplings. This level is discussed here because it was based on a deciduous tree species. For consistency with the approach used for crops, an AOT40 index value was selected. A few

studies have shown that O_3 can be taken up by tree species at nighttime, e.g., young birch trees (Matyssek et al., 1995). However, because most evidence suggests that O_3 deposition at nighttime is low (Coe et al., 1995; Rondon et al., 1993), a value for only daylight hours was selected in Europe (Fuhrer et al., 1997; Kärenlampi and Skärby, 1996). European beech was selected for development of a Level I critical level, because data from several studies were available for this species and because deciduous tree species were judged to be more sensitive to O_3 compared to evergreen tree species (Fuhrer et al., 1997; Kärenlampi and Skärby, 1996). A critical level was defined as an AOT40 value of 10 ppm·h for daylight hours for a 6-month growing season (Kärenlampi and Skärby, 1996). However, other studies have shown that other species such as silver birch may be more sensitive to O_3 than beech (Pääkkönen et al., 1996). Level I critical values are not designed for making quantitative estimates of the O_3 effects on vegetation at the regional scale, instead a so-called Level II critical value is required for this purpose. For long-lived perennials, additional problems complicate extrapolation. As discussed below (Section AX9.5.5.7), considerable scaling is required to extrapolate from experiments conducted with tree seedlings to estimate effects on mature trees in forests. Because of these scaling issues, there is greater uncertainty in estimating effects on forest trees than on annual plants such as crops. While some information is available for addressing issues such as scaling from seedlings to mature trees and estimating O_3 uptake, this information may still be insufficient for developing a Level II approach that can provide quantitative estimates of forest growth losses due to O_3 (Broadmeadow, 1998).

AX9.5.5.4 Summary of Effects on Deciduous Woody Species

Recent evidence from free air exposure systems and OTCs supports results observed previously in OTC studies (Table AX9-15, Figure AX9-18). Specifically, a series of FACE studies undertaken in Rhinelander, WI (Isebrands et al., 2000, 2001) showed that O_3-symptom expression was generally similar in OTCs, FACE, and ambient-O_3 gradient sites, supporting the previously observed variation among aspen clones using OTCs (Karnosky et al., 1999). This study also found no effects on sugar maple growth after 3 years, but in years 4 to 7 found significant growth reduction due to O_3 (Karnosky et al., 2005). These results are important, because they indicate results obtained from OTCs are supported by results from free air exposure systems and also that more than 3 years may be required to adequately investigate the effects of

O_3 on the growth of tree species. Finally, this study found that competition may alter the effect of O_3, depending on environmental conditions and genotype (McDonald et al., 2002). New evidence is also available comparing various aspects of O_3 sensitivity between seedlings and mature trees of some species, notably red oak. As has been observed in previous O_3 criteria documents, root growth is often found to be the most sensitive indicator in terms of biomass response to O_3.

Results since 1996 support the conclusions of the 1996 AQCD (U.S. Environmental Protection Agency, 1996) that individual deciduous trees are generally less sensitive to O_3 than are most annual plants, with the exception of a few genera such as *Populus*, which are highly sensitive. However, the data presented in Table AX9-18 suggest that ambient exposures that occur in different regions of the United States can sometimes reduce the growth of seedlings of deciduous species. Results from multiple-year studies sometimes show a pattern of increasing effects in subsequent years. Although, in some cases, growth decreases due to O_3 become less significant or even disappear over time. While some mature trees show greater O_3 sensitivity in physiological parameters such as net photosynthetic rate compared to seedlings, these effects may not translate into measurable reductions in biomass growth. Because even multiple-year experiments do not expose trees to O_3 for more than a small fraction of their life span and because competition may, in some cases, exacerbate the effects of O_3 on individual species, determining the effects on mature trees remains a significant challenge. Effects on mature trees under natural conditions are discussed after the review of evergreen species below and more fully in Section AX9.6, in the context of extrapolating from controlled studies to forest ecosystems.

AX9.5.5.5 Evergreen Woody Species

Most investigations have shown evergreen tree species to be less sensitive to O_3 compared to deciduous species (U.S. Environmental Protection Agency, 1996). For example, exposure-response functions based on 23 experimental cases of seedling response to O_3, suggest that a SUM06 exposure for 3 months of 42.6 ppm·h would protect evergreen species from a 10% growth loss in 50% of the cases (Table AX9-15). For deciduous species, the corresponding SUM06 value was 31.5 ppm·h (Table AX9-15). As another example, experiments in the Great Smoky Mountains National Park found black cherry seedlings to demonstrate substantial

decreases in biomass, as discussed above and shown in Table AX9-18 (Neufeld et al., 1995). However, exposure for up to three growing seasons did not decrease the biomass of eastern hemlock, Table Mountain pine, and Virginia pine seedlings exposed to O_3 under similar conditions in this location, as shown in Table AX9-19 (Neufeld et al., 2000).

As for deciduous species, there is a substantial range in sensitivity among evergreen species. As discussed above for deciduous species, a risk analysis was undertaken to predict tree biomass growth reductions due to O_3 based on exposure-response equations for tree seedlings combined with species distribution across the eastern United States and interpolated O_3 exposure (Hogsett et al., 1997). While some species such as Virginia pine were predicted to be affected only slightly even in a high O_3 year, the growth of sensitive evergreen species such white pine was predicted to be reduced by 5% in a lower-than-average O_3 year and 10% in a high O_3 year across 50% of its range (Andersen et al., 1997). The remainder of this section discusses experimental results for evergreen species in the order shown in Table AX9-19.

Douglas fir seedlings were exposed to elevated O_3 concentrations in a free air zonal air pollution system in British Columbia, Canada for two growing seasons with 12-h mean values in 1988 of 18 to 41 ppb and in 1989 of 27 to 66 ppb (Runeckles and Wright, 1996). Although substantial variation was seen in effects among the different treatments, there was a significant decrease in the growth of the second flush weight in the second year, with reductions of 55% at the highest O_3 exposure, based on a linear regression. This result contrasts with the lack of effect seen in a previous study with seedlings of this species grown in pots for 134 days and exposed to 7-h mean O_3 concentrations up to 71 ppb (Table 9-30 in U.S. Environmental Protection Agency, 1996).

First-year loblolly pine seedlings of 53 open-pollinated families were exposed to 1×-ambient O_3 in OTCs for a single growing season, and average growth volume was decreased by 14% compared to a CF treatment (McLaughlin et al., 1994). The 1×-ambient O_3 exposure in this study expressed as 24-h SUM00 was 137 ppm·h, and the CF treatment reduced O_3 by 47%. In this study, the root-to-shoot ratio was decreased significantly in six of the nine families examined. Exposure to O_3 with SUM06 values up to 162 ppm·h and 132 ppm·h in 2 successive years in OTCs had no effect on seedlings grown in competition with various species of grasses and forbs (Barbo et al., 2002). Exposure of 3-year-old seedlings to O_3 exposures of up to

2.5×-ambient (12-h mean of 98 ppb) also had no significant effect (Anttonen et al., 1996). Four-week-old loblolly pine seedlings were grown in large OTCs in Alabama and exposed to CF, 1× ambient, 2× ambient O_3 treatments in 2 one year experiments, with seasonal 12-h mean O_3 concentrations of 13, 47, 98 ppm·h in 1998 and 12, 44, 97 ppm·h in 1999 (Estes et al., 2004). Shoot biomass was decreased 15% in the 1× treatment and 22% in 2× in both years, while root biomass was decreased by 26% in the 2× treatment in both years. Foliar symptoms were significantly greater in the 1× treatment iin1999 and in the 2× treatment in both years. Information summarized in the 1996 AQCD (U.S. Environmental Protection Agency, 1996), indicated that significant effects on seedling growth were observed in several studies of seedlings exposed to elevated O_3 concentrations for one or more years. Several studies, including that of McLaughlin et al. (1994), demonstrate considerable variation in O_3 sensitivity among different genotypes of loblolly pine.

For Ponderosa pine seedlings, the 1996 AQCD reviewed a number of studies with exposures to elevated O_3 concentrations for one to three growing seasons (U.S. Environmental Protection Agency, 1996). More recent similar studies support the earlier results (Table AX9-19) (Andersen et al., 2001; Takemoto et al., 1997). The 1996 criteria document also discussed at some length the ongoing work examining effects of O_3 across a naturally-occurring O_3 gradient in the San Bernardino Mountains in California. Since that time, much research on ponderosa pine has focused on interactive effects of additional stresses such as nitrogen and on effects of O_3 on physiological parameters (Sections AX9.3, AX9.6). Effort has also been focused on the effects of O_3 on root growth because such effects could alter sensitivity to drought or nutrient stress. Ecosystem level effects of O_3 are discussed further in Section AX9.6, but some information relevant to exposure-response relationships is discussed below.

For several tree species, O_3 has been shown in experimental studies with seedlings to reduce root growth more than shoot growth (U.S. Environmental Protection Agency, 1996). Ponderosa pine has been shown to be sensitive to O_3, and studies with seedlings have shown reduced root growth, decreases root-to-shoot ratios, and decreased allocation to roots (Andersen et al., 1991, 1997; Andersen and Rygiewicz, 1995; Andersen and Scagel, 1997; Tingey et al., 1976b). Data from a long-studied pollution gradient in the San Bernardino Mountains of southern California suggests that O_3 substantially reduces root growth in natural stands of

ponderosa pine. Root growth in mature trees was decreased at least 87% in a high pollution site as compared to a low pollution site (Grulke et al., 1998a), and a similar pattern was found in a separate study with whole tree harvest along this gradient (Grulke and Balduman, 1999). Because other potential influences on root growth, including shading by competing trees, soil temperature, soil moisture, phenology, were not correlated with the observed pattern of reduced root growth, the authors conclude that O_3 was the cause of the observed decline in root growth. Further results of field investigations with ponderosa pine and other pine species native to California are discussed below under the heading "Scaling experimental data on long-lived species to field conditions" as well as in Section AX9.6.

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

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

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

AX9.5.5.6 Summary of Effects on Evergreen Woody Species

In summary, the O_3 sensitivity of different genotypes within species and between species of evergreen vegetation varies widely. Based on studies with evergreen seedlings in OTCs, major species in the United States are generally less sensitive than are most deciduous trees, and slower-growing species are less sensitive than faster-growing ones. However, exposure to ambient O_3 may reduce the growth of seedlings of commonly occurring species. Because tree species are long-lived, most experiments have only covered a very small portion of the life span of a tree, making estimation of any effect on mature trees difficult. Considerations for scaling the results of seedling studies to mature forest trees as well as additional information from field surveys and studies of mature trees under natural conditions are discussed below and in Section AX9.6.

AX9.5.5.7 Scaling Experimental Data to Mature Trees

As compared with annual crop species, it is much more difficult to define appropriate exposure-response relationships for tree species. For annual species, an experiment may cover the whole life span of the plant, but it is difficult and expensive to provide controlled-exposure conditions for long-lived plants for any significant portion of their life spans, although a few FACE studies have demonstrated that it is feasible. However, FACE studies cannot investigate the effects of ambient O_3 exposures, because lower-than-ambient O_3 treatments cannot be applied. Most studies have used small seedlings, because they are manageable under experimental conditions; but seedlings and mature trees may have different sensitivities to O_3. For perennial species, effects of O_3 may accumulate over more than 1 year, and may interact with other stresses such as drought stress over multiple growing seasons. As for annual species (Section AX9.3.2), substantial variability occurs among evergreen genotypes and this variation may interact with other stress responses differently in different landscapes and regions. Despite these difficulties, investigators have addressed some of these issues since the 1996 AQCD (U.S. Environmental Protection Agency, 1996). New information is available on the response of mature evergreen trees to O_3 under field conditions, and models based on tree physiology and stand dynamics have been used to predict O_3 effects on forest stands and regions. The following issues are reviewed briefly below: (1) interaction of drought and O_3 stress, (2) scaling data from

seedlings to mature-tree studies. Two additional scaling issues are addressed in Section AX9.6: (1) scaling data to forest stands, and (2) scaling data to ecosystems and regions.

AX9.5.5.7.1 Interactive Effects of Drought and Ozone

Many interacting factors may influence the effect of O_3 on vegetation. For crop plants, environmental conditions are often managed such that nutrients and water are not strongly limiting; but for native vegetation, including most perennial species, such factors are likely to limit growth. The effects of interacting stresses on vegetation were reviewed in Section AX9.3. However, because drought is common in many forests, and because there is substantial evidence that it alters the response of trees to O_3, it is discussed in this section in the context of determining exposure-response relationships for trees.

Controlled experiments with seedlings provide direct evidence that drought can reduce the impact of O_3. For example, for 3-year-old Norway spruce in Sweden, exposure to elevated O_3 for three growing seasons decreased total biomass by 18% and stem biomass by 28% (Karlsson et al., 1995). However, for droughted trees, both total and stem biomass decreased only 5%, with a statistically significant interaction with O_3 for stem biomass. Yet after a fourth season of exposure, there was no longer any interaction between drought and O_3, while there was a significant decrease of 8% in the biomass when both drought and well-watered data were combined (Karlsson et al., 2002). In this study, seedlings were grown in sand in 120-L pots and for the drought treatment, water was withheld for 4 weeks during the first year and for 7 to 8 weeks during each of the last 3 years. In this experiment, the O_3 exposures expressed as 12-h seasonal daylight mean averaged over four growing seasons were 12 and 44 ppb for the CF and 1.5×-ambient treatments, respectively. Over this period, the AOT40 values for the treatments averaged 2 and 23 ppm·h respectively. Despite 4 years of exposures, this experiment did not demonstrate a consistent trend in drought O_3 interactions. The difference in effects seen between the third and fourth season suggest that scaling drought-O_3 interactions from seedlings to mature trees may be difficult. However, evidence from biomonitoring surveys supports an interaction between drought and O_3 effects, at least for foliar symptoms. In systematic surveys of foliar symptoms on species selected as biomonitors throughout much of the eastern United States, symptoms were more common and more severe in areas with high O_3 concentrations (Smith

et al., 2003). However, in very dry years, such as 1999, the occurrence and severity of symptoms was greatly reduced, even in areas with high ambient O_3 concentrations.

AX9.5.5.7.2 Scaling from Seedlings to Mature Trees

Because most experiments are conducted with seedlings, various methods are required to scale experimental data on seedlings to mature trees. An overview of physiological differences between young and old plants, and the consequences of these differences for O_3 sensitivity, was provided in Section AX9.3.5.3. The discussion below focuses on information relevant to developing exposure-response relationships for mature trees. Information from a few experimental studies, as well as scaling efforts based on physiological characteristics incorporated into models, are discussed in Section AX9.6.

Although most studies continue to examine the effects of O_3 on seedlings, during the 1990s some studies examined the effects of O_3 on the response of mature trees. Studies of mature trees demonstrate differences in some aspects of O_3 sensitivity between seedlings and mature trees. For some species, such as red oak, seedlings are less sensitive to O_3 than are mature trees (Hanson et al., 1994; Samuelson and Edwards, 1993; Wullschleger et al., 1996). Both red oak seedlings and genetically related mature trees were exposed to CF, 1×-ambient, or 2×-ambient O_3 exposures in OTCs in Tennessee for two growing seasons (Hanson et al., 1994). Nine large chambers (4.6 × 8.2 m) were used to enclose individual mature trees and standard EPA-style OTCs were used for potted seedlings. Ozone exposures expressed as a 24-h SUM00 were 34, 79, and 147 ppm·h in 1992 and 37, 95, 188 ppm·h in 1993 for the sub-ambient, and 2×-ambient treatments. Mature trees had a greater light-saturated net photosynthetic rate and stomatal conductance compared to seedling foliage at physiological maturity. By the end of the growing season, exposure to 1×-ambient and 1×-ambient O_3 reduced the light-saturated net photosynthetic rate and stomatal conductance of mature trees by 25 and 50%, respectively, compared with the CF treatment (35 ppm·h). In seedlings, however, light-saturated net photosynthetic rate and stomatal conductance were less affected by O_3 exposure. The authors concluded that extrapolations of the results of seedling-exposure studies to foliar responses of mature forests without considering differences in foliar anatomy and stomatal response between juvenile and mature foliage may introduce large errors into projections of the O_3 responses of mature trees.

In a study of ponderosa pine in California, seedlings and branches of mature trees (in branch chambers) were exposed to O_3 concentrations of 0.5-, 1-, and 2×-ambient O_3 concentrations (Momen et al., 1997). Net photosynthetic rate of 1-year-old, but not current-year, foliage was reduced in mature trees but not significantly reduced in seedlings. This effect was not due to alteration of stomatal conductance by O_3. This result contrasts with those with earlier studies of red spruce (Rebbeck et al., 1993).

In contrast to the findings for red oak and Ponderosa pine, giant sequoia seedlings had higher rates of stomatal conductance, CO_2-exchange rate, and dark respiration than did mature trees (Grulke and Miller, 1994). As compared to older trees, stomatal conductance was more than 7-fold greater in current-year, and 4-fold greater in 2-year-old, seedlings (Grulke and Miller, 1994). The authors concluded that giant sequoia seedlings are sensitive to atmospheric O_3 until ~5 years of age. Low conductance, high water use efficiency, and compact mesophyll all contribute to a natural O_3 tolerance, or to O_3 defense, or to both, in the foliage of older trees. Similarly, lower stomatal conductance was found in mature Norway spruce in Austria compared to seedlings grown with optimal water and nutrients in a growth chamber (Wieser, 1997). In this study, net photosynthetic rate was less sensitive to added O_3 in mature trees compared to seedlings. In a related study, the average rate of O_3 uptake of 17-year-old trees ~0.6 nmol m^{-2} s^{-1}, decreasing linearly in older trees, such that rates were only ~0.1 m^{-2} s^{-1} in 216-year-old trees (Wieser et al., 1999).

Based on a review of studies of stomatal conductance in both seedlings and mature trees, Samuelson and Kelly (2001) concluded that O_3 uptake in oak species, black cherry, sugar maple, and American beech averaged 47% lower in potted seedlings than in mature trees. For evergreen species, they concluded that O_3 uptake in seedlings averaged 26% higher than in mature trees. They also suggested that artifacts introduced by growth in pots confound these differences that exposure-response functions derived from seedlings grown in situ are more applicable to mature trees than are studies of seedlings grown in pots (Samuelson and Kelly, 2001).

As discussed above for annual vegetation, it has long been noted that internal O_3 dose is more appropriate than external O_3 exposure for assessing the effects of O_3 on vegetation, because effects occur primarily via the uptake of O_3 through the stomata (Section AX9.2.2). However, external O_3 exposure sometimes has been shown to explain O_3 effects as well or better than calculated internal O_3 dose. For ponderosa pine, Grulke and others (2002b) found little

difference in the response of net photosynthetic rate and stomatal conductance to O_3 exposure as compared to calculated O_3 uptake; and estimated O_3 uptake by ponderosa pine and O_3 exposure at several sites were highly correlated ($r^2 = 0.92$). For red oak, Hanson and others (Hanson et al., 1994) found that SUM00 explained 83% of the variance in the response of light-saturated photosynthetic rate to O_3 levels, while estimated internal dose explained only 76% of the variance. In this same study, SUM06 explained only 49% of the variance. Due to genetic variation or other factors, individual mature trees will vary in their response to similar O_3 exposures. For example, in 125-year-old giant sequoia trees exposed to ~230 ppm·h of O_3 in branch chambers, O_3 uptake in one individual was ~5 mmol m^{-2}, while in another it was ~9.5 mmol m^{-2} (Grulke et al., 1996).

Based on these results, stomatal conductance, O_3 uptake, and O_3 effects cannot be assumed to be equivalent in seedlings and mature trees. In general, mature deciduous trees are likely to be more sensitive to O_3 compared to seedlings, while mature evergreen trees are likely to be less sensitive than seedlings. However, even when differences in physiological traits occur, concomitant effects on stem growth may not be detected in the field. Additionally, complex interactions may occur between environmental conditions and O_3 responses; and artifacts may occur for seedling studies, especially for seedlings grown in pots. Finally, competition between species or genotypes within a species can either exacerbate or ameliorate the effects of O_3. Such effects are predicted by models of the growth of mixed species forests, as discussed in Section AX9.6, and various interactions between competitive ability and O_3 effects have been found for aspen clones in the Wisconsin FACE study (McDonald et al., 2002). Section AX9.6 further discusses issues that must be addressed when scaling data from individual mature trees to forests and regions.

AX9.5.6 Studies With the Chemical EDU

The chemical EDU has been used with the goal of protecting plants from O_3 effects without controlling O_3 exposure (Table AX9-21) (U.S. Environmental Protection Agency, 1986, 1996). As discussed in Section AX9.1.3.3, the use of EDU has the potential to be a low-cost, practical method of evaluating ambient O_3 exposures on plants grown under natural conditions without the limitations imposed by methodologies such as OTCs (Section AX9.1.3.3). However, because EDU is phytotoxic, and may have effects on plants other than antioxidant protection,

Table AX9-21. 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 et 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 AX9-21 (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 (2002a)
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 of150 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)

it is crucial that the correct dosage for protection from O_3 be determined without the direct effects of EDU. For example, a study in Massachusetts applied EDU to foliage of field-grown bush beans of two lines (S156, R123) and two cultivars (Bush Blue Lake 290, Bush Blue Lake 274) (Elagöz and Manning, 2005a). EDU increased the above-ground biomass of one line but decreased the above-ground biomass in the other line and in one of the cultivars. Other studies have shown that EDU does not always have greater effects at higher O_3 exposures (Ribas and Penuelas, 2000; Tonneijck and Van Dijk, 1997). Such results suggest that it may be difficult to quantify ambient O_3 effects using EDU, because the amount of plant growth or yield expected at a low (background) O_3 concentration cannot be inferred from EDU-treated plants grown at locations with higher O_3 exposures. Unfortunately, although many studies with EDU have been conducted in recent decades, very few have used multiple EDU application levels along with multiple O_3 exposures to characterize the EDU system for a given plant species. Therefore, the text of this section focuses on how data from existing studies can be used for developing or validating exposure-response relationships, rather than reviewing results of all individual studies. Data from individual studies on O_3 exposure, EDU application rates, and the effects of EDU are presented in Table AX9-21. In addition to EDU, sodium erythorbate has been used in a few studies as a protectant chemical. Since very few published studies have used sodium erythorbate and attempts to establish appropriate doses for individual species are even more limited, the use of this chemical is not reviewed here.

In summary, it is difficult to use data from existing EDU studies to develop exposure-response relationships or to quantify the effects of ambient O_3 exposure. Despite these limitations, the EDU studies reviewed in previous criteria documents (U.S. Environmental Protection Agency, 1986, 1996) and the more recent studies summarized in Table AX9-21 provide another line of evidence that ambient O_3 exposures occurring in many regions of the United States are reducing the growth of crops and trees.

AX9.5.7 Summary

Data published during the last decade support the conclusions of previous criteria documents that there is strong evidence that ambient concentrations of O_3 cause injury and damage to numerous common and economically valuable plant species. For annual vegetation, the data summarized in Table AX9-16 show a range of growth and yield responses both within

and among species. Nearly all of these data were derived from studies in OTCs, with only two studies using open-air systems in the UK (Ollerenshaw et al., 1999; Ollerenshaw and Lyons, 1999). It is difficult to compare studies that report O_3 exposure using different indices, such as AOT40, SUM06, or 7-h or 12-h mean values. However, when such comparisons can be made, the results of more recent research confirm earlier results summarized in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). A summary of earlier literature concluded that a 7-h, 3-month mean of 49 ppb corresponding to a SUM06 exposure of 26 ppm·h would cause 10% loss in 50% of 49 experimental cases (Tingey et al., 1991). More recent data summarized in Table AX9-16 support this conclusion, and more generally indicate that ambient O_3 exposures can reduce the growth and yield of annual species. Some annual species such as soybean are more sensitive, and greater losses may be expected (Table AX9-16). A two-year study using a free-air exposure system with soybean confirmed yield reductions found previously using OTCs (Morgan et al., in press). Thus, the more recent scientific literature supports the conclusions of the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) that ambient O_3 concentrations are probably reducing the yield of major crops in the United States.

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

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

The use of OTCs may reverse the usual vertical gradient in O_3 that occurs within a few meters above the ground surface (Section 3.3.2, Section AX9.1). This reversal suggests that OTC studies may somewhat overestimate the effects of an O_3 concentration measured several meters above the ground. However, such considerations do not invalidate the conclusion of the 1996 AQCD (U.S. Environmental Protection Agency, 1996) that ambient O_3 exposures (Tables AX9-13 and AX9-21) are sufficient to reduce the yield of major crops in the United States. Recent studies using OTCs confirmed previous results that ambient ozone exposures can significantly reduce the growth of annual species and the yield of commercially important crop species. Additionally, a two-year study using a free air exposure system with soybean confirmed yield reductions found previously using OTCs (Morgan et al, in press).

As for single-season agricultural crops, yields of multiple-year forage crops are reduced at O_3 exposures that occur over large areas of the United States. This result is similar to that reported in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). When species are grown in mixtures, O_3 exposure can increase the growth of O_3-tolerant species and exacerbate the growth decrease of O_3-sensitive species (e.g., Ashmore and Ainsworth, 1995; Fuhrer et al., 1994). Because of this competitive interaction, the total growth of the mixed-species community may not be affected by O_3 exposure (Ashmore and Ainsworth, 1995; Barbo et al., 1998; Fuhrer et al., 1994). However, in some cases, mixtures of grasses and clover species have shown significant decreases in total biomass growth in response to O_3 exposure in studies in the United States (Heagle et al., 1989; Kohut et al., 1988) and in Sweden (Pleijel et al., 1996). In Europe, a provisional critical level for herbaceous perennials of an AOT40 value of 7 ppm·h over 6 months has been proposed to protect sensitive plant species from adverse effects of O_3.

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

effects of O_3 on the growth of tree species. As has been observed in previous criteria documents, root growth often is found to be the most sensitive biomass response indicator to O_3.

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

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

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

For all types of perennial vegetation, cumulative effects over more than one growing season may be important; furthermore, studies for only a single season may underestimate effects. Mature trees may be more or less sensitive to O_3 than are seedlings, depending on the

species, but information on physiological traits may be used to predict some such differences. In some cases, mature trees may be more sensitive to O_3 than seedlings due to differences in their gas exchange rates, growth rates, greater cumulative exposures, or due to the interaction of O_3 stress with other stresses.

AX9.6 EFFECTS OF OZONE EXPOSURE ON NATURAL ECOSYSTEMS

AX9.6.1 Introduction

The preceding section on species-level responses (AX9.5) provides a lead-in to address the response of ecosystems to O_3. The conclusion of the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) was that aside from the results from the San Bernardino NF, there was no direct evidence that O_3 is altering natural ecosystems in the United States. This conclusion is generally valid today, except that our understanding of the effects of O_3 in the San Bernardino NF has been tempered by additional understanding of the complicating role that N deposition plays in this system. Despite the lack of any new, direct information linking O_3 with ecosystem changes, numerous publications since 1996 have highlighted ways in which O_3 may affect ecosystem structure and/or function. This section addresses new and (where appropriate) older literature in order to illustrate possible shifts in energy or material flow through ecosystems as a result of O_3 exposure.

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

Each level of organization within an ecosystem has functional and structural characteristics. At the ecosystem level, functional characteristics include, but are not limited, to energy flow; nutrient, hydrologic, and biogeochemical cycling; and maintenance of food chains.

The sum of the functions carried out by ecosystem components provides many benefits to mankind, as in the case of forest ecosystems (Smith, 1992). These include food, fiber production, aesthetics, genetic diversity, and energy exchange.

Ecosystems are functionally highly integrated. Changes in one part of an ecosystem, such as the primary producer component, may have consequences for connected parts, such as the consumer and decomposer components. For example, when needles are shed prematurely as a result of O_3 exposure, successional development of phyllosphere fungi inhabiting the surface of Ponderosa pine needles may be truncated (Bruhn, 1980). In addition, decomposer populations in the litter layer may be capable of higher rates of decomposition, due to the higher N content of the younger age classes of needles falling from O_3-damaged pines (Fenn and Dunn, 1989). Because ecological systems integrate the effects of many influences, the results of O_3 exposure may depend on co-occurring influences that predispose an ecosystem to stress (Colls and Unsworth, 1992). One important change in our thinking since the 1996 O_3 AQCD is that, at the high levels of O_3 exposure that are known to result in detectable plant responses (>250 ppm h accumulated over a growing season), N deposition must also be considered as a concurrent stressor. Both O_3 exposure and increased N deposition can cause changes in N cycling and compartmentalization within ecosystems.

The vast majority of O_3-effects literature addresses individual species responses (see Section AX9.6.4.3), as was also true in 1996 (U.S. Environmental Protection Agency, 1996). This section differs from the preceding one in that the physiological stress of individual species is considered only within the context of its natural ecosystem. Changes in function at the individual level propagate through the higher levels of organization, resulting in changes in ecosystem structure and function. However, since ecosystem-level responses result from the interaction of organisms with one another and with their physical environment, it takes longer for a change to develop to a level of prominence at which it can be identified and measured. The paucity of scientific literature on O_3 effects at the ecosystem level is a result of both the complexity of ecological systems, and long response times. In addition, "indirect" effects of O_3 on plants (e.g., effects that alter the plants' ability to integrate environmental stresses) may be more important than the direct effects on photosynthesis and respiration at the leaf level (Johnson and Taylor, 1989).

A conceptual framework (see Table AX9-22) for discussing the effects of O_3 on ecosystems was developed by the EPA Science Advisory Board (Young and Sanzone, 2002). Their six essential ecological attributes (EEAs) include landscape condition, biotic condition, organism condition, ecological processes, hydrological and geomorphological processes, and natural disturbance regimes (see Table AX9-22). The major ecological effects of O_3, and gaps in our knowledge of O_3 exposure effects at the ecosystem level, are summarized at the end of this chapter. While the main focus is O_3 effects newly described since the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), many key historical papers are cited to demonstrate ecosystem response, particularly where they remain the only examples in the literature. Although the vast majority of published studies focus on individuals, six case studies (five field examples and one FACE experiment) have measured several ecosystem components simultaneously to better understand the overall ecosystem response to O_3. We provide an overview of these six studies up-front to provide a context for the subsequent discussion on possible ecosystem effects on an EEA basis.

AX9.6.2 Case Studies

AX9.6.2.1 Valley of Mexico

The first evidence of air pollution impacts on vegetation in the Valley of Mexico (Mexico City Air Basin) were observations of foliar injury symptoms in bioindicator plants attributed to O_3, PAN, SO_2, and possibly other pollutants (De Bauer, 1972). Subsequently, O_3 injury to foliage and crowns of pine trees were reported in forests to the south and southwest of Mexico City (De Bauer and Hernández-Tejeda, 1986; De Bauer and Krupa, 1990). Ozone is considered to be the pollutant with the most severe impacts on vegetation within the Mexico City urban zone and in forests downwind of the city. *Pinus hartwegii* is the most O_3-sensitive pine species and is severely impacted by high O_3 exposures encountered to the south/southwest of the Mexico City metropolitan area (Miller et al., 1994). The potential for O_3 injury is particularly high in this area, because O_3 levels are high during the summer rainy season when soil moisture availability and stomatal conductance are greatest; these factors enhance O_3 uptake and injury. Decline of *Abies religiosa* (oyamel) in the Desierto de los Leones NP is a well-known example of dramatic dieback and mortality of entire forest stands due primarily to air pollution stress (Alvarado et al., 1993). Other factors, such as a lack of stand thinning, also contribute to forest

Table AX9-22. Essential Ecological Attributes for Natural Ecosystems Affected by O_3

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	*Pinus ponderosa*	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)
	Populus tremuloides and *Betula papyrifera*	Soil microbial community	Phillips et al. (2002)
	Pinus ponderosa	Soil microbial community	Scagel and Andersen (1997)
	Pinus taeda	Fungal morphotypes	Edwards and Kelly (1992); Qui et al. (1993)
Trophic Interactions *Insects*	*Pinus ponderosa*	Bark beetle severity	Cobb et al. (1968)
	Pinus ponderosa	Bark beetle productivity and predator/parasitoid density	Dahlsten et al. (1997)
	Populus tremuloides	Blotch leaf miner performance	Kopper and Lindroth (2003a)
	Populus tremuloides	Aphid/natural enemy abundance	Percy et al. (2002)
	Populus tremuloides	Forest tent caterpillar/ paratisoid performance	Percy et al. (2002); Holton et al. (2003)
Diseases	*Populus hybrids*	*Septoria* occurrence	Woodbury et al. (1994)
	Populus hybrids	Rust occurrence	Beare et al. (1999a)
	Populus tremuloides	Rust occurrence	Karnosky et al. (2002)
	Picea abies and *Picea sitchensis*	Needle fungi	Magan et al. (1995)
	Pinus ponderosa	Root disease × O_3 interactions	Fenn et al. (1990)
	Pinus taeda	Canker dimensions	Carey and Kelley (1994)
	Pinus sylvestris/ mycorrhizae	Disease susceptibility	Bonello et al. (1993)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O_3

Category	Species	Condition Measures	References
Community Dynamics	*Pinus ponderosa/Abies concolor/Calocedrus decurrens*	Abundance	Minnich et al. (1995)
	Populus tremuloides	Competitive status	McDonald et al. (2002)
	Pinus ponderosa/ Elymus glaucus	O_3 sensitivity	Andersen et al. (2001)
	Pinus taeda/diverse community	Tree growth	Barbo et al. (2002)
• Species and Populations			
Population Size	*Pinus strobus*	Mortality	Karnosky (1981)
	Pinus ponderosa	Mortality	Carroll et al. (2003)
Genetic Diversity/ Population Structure	*Lupinus bicolor*	% population sensitive	Dunn (1959)
	Populus tremuloides	% population sensitive	Berrang et al. (1986, 1989, 1991)
	Trifolium repens	% population sensitive	Heagle et al. (1991)
	Plantago major	% population sensitive	Davison and Reiling (1995); Reiling and Davison (1992b); Lyons et al. (1997)
Population Dynamics	*Trifolium repens*	Adaptation	Heagle et al. (1991)
	Plantago major	Population changes over time	Davison and Reiling (1995)
Organism Condition			
• Visible Symptoms	*Pinus ponderosa*	Foliar symptoms	Grulke and Lee (1997); Arbaugh et al. (1998); Salardino and Carroll (1998); Temple et al. (1992) Grulke et al. (2003)
	Pinus jeffreyi	Foliar symptoms	Patterson and Rundel (1995); Salardino and Carroll (1998); Fredericksen et al. (1995, 1996b); Chappelka et al. (1997, 1999a,b)
	Prunus serotina	Foliar symptoms	Hildebrand et al. (1996); Ghosh et al. (1998); Lee et al. (1999); Ferdinand et al. (2000); Schaub et al. (2003)
	Liriodendron tulipfera	Foliar symptoms	Yuska et al. (2003); Somers et al. (1998); Hildebrand et al. (1996)
	Sassafras albidum	Foliar symptoms	Chappelka et al. (1999a,b)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O_3

Category	Species	Condition Measures	References
Organism Condition (cont'd)			
• Visible Symptoms (cont'd)	*Populus nigra, Fraxinus excelsior* and *Prunus avium*	Foliar symptoms	Chappelka et al. (1999a); Novak et al. (2003)
	Fagus sylvatica	Foliar symptoms	Gerosa et al. (2003); Vollenweider et al. (2003b)
	Fraxinus americana	Foliar symptoms	Schaub et al. (2003)
	Grassland species	Foliar symptoms	Bungener et al. (1999a)
	Herbaceous species	Foliar symptoms	Bergmann et al. (1999)
	Asclepias exaltata	Foliar symptoms	Chappelka et al. (1997)
	Rudbeckia laciniata and *Verbesina occidentalis*	Foliar symptoms	Chappelka et al. (2003)
	Asclepias incarnata	Foliar symptoms	Orendovici et al. (2003)
• Physiological Status	*Pinus halepensis*	Allometry	Wellburn and Wellburn (1994)
	Populus tremuloides	Crown architecture	Dickson et al. (2001)
	Betula pendula	Crown architecture	Kull et al. (2003)
	Fagus sylvatica	Crown architecture	Stribley and Ashmore (2002)
	Populus tremuloides	Root dry weight	Coleman et al. (1996)
	Pinus ponderosa	Root/shoot ratio	Grulke et al. (1998a); Grulke and Balduman (1999)
	Festiva ovina	Root/shoot ratio	Warwick and Taylor (1995)
	Betula pubescens	Root/shoot ratio	Mortensen (1998)
	Populus tremuloides × *Populus tremula*	Root/shoot ratio	Landolt et al. (2000); Paludan-Müller et al. (1999)
	Populus tremuloides	Leaf area index	Oksanen et al. (2001)
	Pinus ponderosa	Carbon allocation to mycorrhizae	Neufeld et al. (1995); Wiltshire et al. (1996); Andersen and Rygiewicz (1995a,b)
	Betula pendula	Decreased winter bud formation	Karnosky et al. (2003a)
	Betula pendula	Delayed bud break	Oksanen (2003a,b)
	Acer saccharum	Early bud break	Prozherina et al. (2003); Bertrand et al. (1999)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O_3

Category	Species	Condition Measures	References
• Reproductive Status	*Apocynun androsaemifolium*	Flowering time	Bergweiler and Manning (1999)
	Buddleia davidii	Flowering time	Findley et al. (1997)
	Rubus cuneifolius	Pollen germination	Chappelka (2002)
	Plantago major	Pollen tube elongation	Stewart (1998)
	Fragaria × ananassa	Fruit yield	Drogoudi and Ashmore (2000, 2001)
	Plantago major	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)
Ecological Processes			
• Energy Flow			
Primary Production	*Pinus ponderosa*	Photosynthesis	Miller et al. (1969); Clark et al. (1995); Takemoto et al. (1997); Grulke et al. (2002b)
	Pinus ponderosa	Needle retention	Temple et al. (1993)
	Populus tremuloides	Photosynthesis	Coleman et al. (1995ab); Noormets et al. (2001a,b); Sharma et al. (2003); Karnosky et al. (2003a); Oksanen (2003a,b)
	Betula pendula	Photosynthesis/conductance	Matyssek et al. (2002)
	Betula pendula	Stem respiration and radial growth	Kelting et al. (1995)
	Quercus rubra	Root turnover	Coleman et al. (1996)
	Populus tremuloides	Soil respiration	King et al. (2001); Andersen and Scagel (1997); Coleman et al. (1995a)
	Pinus ponderosa	Soil respiration	Scagel and Andersen (1997); Andersen (2000); Samuelson and Kelly (1996)
	Quercus rubra	Carbon partitioning and allocation	Andersen et al. (1997); Grulke et al. (1998a)
	Populus tre muloides	Carbon allocation	Grulke and Balduman (1999)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O_3

Category	Species	Condition Measures	References
Primary Production (cont'd)	*Pinus ponderosa*	Carbon allocation	Grulke et al. (2001)
	Betula pendula	Carbon allocation	Karlsson et al. (2003b); Oksanen and Saleem (1999); Saleem et al. (2001)
	Fragaria vesca	Carbon allocation	Manninen et al. (2003)
	Pinus taeda	Root respiration	Edwards (1991)
	Lespedeza cuneata and *Schizacbyrium scoparium*	Yield	Powell et al. (2003)
	Liriodendron tulipfera	Radial growth	Somers et al. (1998); Vollenweider et al. (2003a)
	Prunus serotina	Radial growth	Somers et al. (1998)
	Pinus jeffreyi	Radial growth	Peterson et al. (1987)
	Pinus ponderosa	Radial growth (no effect)	Peterson et al. (1993)
	Pinus strobus	Radial growth	Bartholomay et al. (1997)
	Pinus taeda	Radial growth	McLaughlin and Downing (1995; 1996)
	Fagus sylvatica	Stem volume	Braun et al. (1999)
	Picea abies	Stem volume	Wallin et al. (2002)
	Populus tremuloides	Volume growth	Isebrands et al. (2001)
	Pinus ponderosa	Root growth	Andersen et al. (1991)
Net Ecosystem Production	Northern hardwoods	NPP estimates	Laurence et al. (2000)
	Northern hardwoods	Biomass estimates	Hogsett et al. (1997)
Growth Efficiency	*Plantago major*	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)
	Populus tremuloides	Relative growth rate	Yun and Laurence (1999a)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O_3

Category	Species	Condition Measures	References
Growth Efficiency (Cont'd)	*Fagus sylvatica*	Relative growth rate	Bortier et al. (2000c)
	Picea abies	Relative growth rate	Karlsson et al. (2002)
	Prunus serotina	Relative growth rate	Lee et al. (2002)
• Material Flow *Organic Carbon Cycling*	*Populus tremuloides* and Betula papyrifera	Altered foliar C:N ratio and N resorption efficiency	Lindroth et al. (2001)
	Andropogon virginicus and *Rubus cuneifolius*	Litter decomposition rate	Kim et al. (1998)
	Liriodendron tulipera	Litter decomposition rate	Scherzer et al. (1998)
	Populus deltoides	Litter decomposition rate	Findlay and Jones (1990)
	Pinus ponderosa	Litter decomposition rate	Fenn and Dunn (1989)
	Pinus sylvestris	Litter decomposition (no effect)	Kainulainen et al. (2003)
Nitrogen Cycling	*Pinus ponderosa*	Altered foliar N	Momen and Helms (1996)
	Pinus ponderosa	Foliar N and O_3 exposure no effects	Bytnerowicz et al. (1990)
	Pinus taeda	Altered foliar N metabolism	Manderscheid et al. (1992)
	Prunus serotina and *Liriodendron tulipfera*	Altered foliar N	Boerner and Rebbeck (1995)
Other Nutrient Cycling	*Picea sitchensis* and *Pinus sylvestris*	Foliar leaching no effect	Skeffington and Sutherland (1995)
	Pinus ponderosa	Nutrient availability and O_3	Bytnerowicz et al. (1990)
Hydrology and Geomorphology			
• Water Budget	*Picea rubens*	Water-use efficiency no effect	Laurence et al. (1997)
	Pinus armandi	Water-use efficiency	Shan et al. (1996)
	Pinus jeffreyi	Canopy transpiration	Grulke et al. (2003a)
	Picea abies	Transpiration (xylem sap flow)	Maier-Maercker (1997)
	Fraxinus excelsior	Water stem flow	Wiltshire et al. (1994)
	Betula pendula	Water-use efficiency	Maurer and Matyssek (1997)
	Populus hybrids	Water-use efficiency	Reich and Lassoie (1984)

Table AX9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O_3

Category	Species	Condition Measures	References
Natural Disturbance Regimes			
• Frequency	*Pinus ponderosa*	Frequency of fire	McBride and Laven (1976); Minnich et al. (1995); Miller and McBride (1999)
	Pinus ponderosa	Occurrence of bark beetle outbreaks	Pronos et al. (1999); Dahlsten et al. (1997)
• Intensity	*Picea sitchensis*	Winter damage	Lucas et al. (1988)
	Pinus halepensis	Reduced winter damage	Wellburn and Wellburn (1994)
	Picea rubens	Freezing tolerance	Waite et al. (1994)
	Fagus sylvatica	Drought stress	Pearson and Mansfield (1993, 1994)
	Picea abies	Drought stress	Maier-Maercker (1998); Maier-Maercker and Koch (1992)
	Pinus ponderosa	Fire intensity	Miller and McBride (1999a)
• Extent	*Pinus ponderosa*	Extent of bark beetle attack	Minnich et al. (1995)
• Duration	*Pinus ponderosa*	Duration of bark beetle attack	Minnich et al. (1995)

decline. Lead in automobile gasoline was phased out in 1990, and foliar concentrations of heavy metals in forest species are not now at phytotoxic levels (Fenn and De Bauer, 1999). Sulfur dioxide concentrations decreased in the early 1990s as a result of regulatory mandates limiting their emissions. Sensitive plants in the northeast and northwest sectors of the Mexico City urban zone where concentrations are highest may still be impacted by exposure to ambient SO_2 levels. Deposition of ionic forms of N and S are high in forested areas southwest of Mexico City. However, the effects of these chronic nutrient inputs to the forest are only beginning to be investigated and understood.

The ecological perturbations caused by severe air pollution exposures in forests located downwind of Mexico City are expected to continue for the near future (the next 5 to 10 years), largely as a result of high O_3 concentrations as well as emissions of N oxides. The longer-term response is more uncertain and depends largely on the effectiveness of regulatory emissions control strategies. Currently, pollutant levels are declining. Forest responses to this trend will

depend on how long it takes to reduce levels sufficiently to allow sensitive species to recover. Some of the change to the ecosystem is probably irreversible, such as the loss of lichen diversity and the loss of other O_3-sensitive species (Zambrano and Nash, 2000).

AX9.6.2.2 San Bernardino Mountains

The San Bernardino Mountains lie east of the Los Angeles Air Basin in California, and significant levels of pollution have been transported into the mountain range, including into a Class I wilderness area. The effects of O_3 exposure on the mixed conifer forest of the San Bernardino Mountains is perhaps the longest and most thoroughly documented O_3 ecological effects evaluation (Miller and McBride, 1999a). In this classic case study linking tropospheric O_3 exposure to damage to an entire forest ecosystem (U.S. Environmental Protection Agency, 1996) (Table AX9-23), Miller et al. (1963) first identified the unique foliar chlorotic mottle that was occurring on two of the dominant tree species, *Pinus ponderosa* and *P. jeffreyi*. Levels of O_3 averaging 100 to 120 ppb over 24 h with 1-h peaks well into the 200 ppb range were common in the region in the 1960s and 1970s (Miller and McBride, 1999a). Single-hour peak values have declined in recent years due to heavily regulated pollution control (Arbaugh et al., 1998; Lee et al., 2003; Takemoto et al., 2001).

Since the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) was written, the concurrent role of N deposition in modifying ecosystem response to O_3 exposure in the San Bernardino Mountains has been further elucidated (Arbaugh et al., 2003; Bytnerowicz et al., 1999; Bytnerowicz, 2002; Fenn et al., 1996; Takemoto et al., 2001). Both O_3 exposure and N deposition reduce foliar retention (Grulke and Balduman, 1999) and alter tissue chemistry of both needles and litter (Poth and Fenn, 1998). In addition, confounding factors such as drought and fire suppression add to the complexity of ecosystem response (Arbaugh et al., 2003; Minnich et al., 1995; Takemoto et al., 2001). Extensive crown injury measurements have also been made, linking ambient O_3 exposure data to chlorotic mottle and fascicle retention (Arbaugh et al., 1998). Ozone exposure and N deposition reduce carbon allocation to stems and roots (Grulke et al., 1998a, 2001), further predisposing trees to drought stress, windthrow, root diseases, and insect infestation (Takemoto et al., 2001). Increased mortality of susceptible tree species (Ponderosa and Jeffrey pine) has shifted community composition toward white fir and incense-cedar (*Abies concolor*, *Calocedrus decurrens*) and has altered forest stand structure

Table AX9-23. Case Studies Demonstrating the Ecological Effects of O_3

Study	Keystone Species	Study Type	Period Studied	Key Ecological Findings
Valley of Mexico	*Pinus hartwegii, Abies religiosa*	Field transects	35 yrs	• Significant foliar injury (De Bauer, 1972; De Bauer and Hernández-Tejeda, 1986; De Bauer and Krupa, 1990) • Community composition changes (Alvarado et al., 1993) • Species richness changes (Zambrano and Nash, 2000)
San Bernardino Mountains	*Pinus ponderosa, P. jeffreyi*	Field transects	40 yrs	• Community composition changes (Arbaugh et al., 2003; Miller, 1973; Minnich et al., 1995) • Population changes (McBride and Laven, 1999) • O_3-pine-bark beetle interaction (Pronos et al., 1999) • Altered C flows (Arbaugh et al., 1999; Grulke et al., 1998a, 2001, 2002b; Grulke and Balduman, 1999) • Interaction of O_3, drought, N deposition (Fenn et al., 1996; Grulke, 1999; Takemoto et al., 2001) • Altered carbon cycling (Arbaugh et al., 1999)
Sierra Nevada Mountains	*Pinus ponderosa, P. jeffreyi*	Field	35 yrs	• Wide-scale nature of effects (Edinger et al., 1972; Miller and Millecan, 1971) • Link to decreased growth (Peterson et al., 1987, 1991, 1995) • Quantification of O_3 flux (Bauer et al., 2000; Goldstein et al., 2003; Panek et al., 2002) • Cumulative O_3 effects (Takemoto et al., 1997) • Canopy level responses (Grulke et al., 2003a,b) • Population changes (Carroll et al., 2003)
Appalachian Mountains	*Fraxinus americana, Liriodendron tulipfera, Pinus strobus, Prunus serotina*	Field	25 yrs	• Link of visible symptoms to growth decreases (McLaughlin et al., 1982; Somers et al., 1998) • Wide-scale nature of effects (Chappelka et al., 1999a; Hildebrand et al., 1996)

Table AX9-23 (cont'd). Case Studies Demonstrating the Ecological Effects of O_3

Study	Keystone Species	Study Type	Period Studied	Key Ecological Findings
Aspen FACE	*Acer saccharum, Betula papyrifera, Populus tremuloides*	Open-air O_3 exposure	6 yrs	• Competitive interactions (McDonald et al., 2002) • O_3-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 CO_2 responses by O_3 (Isebrands et al., 2001; Karnosky et al., 2003b; McDonald et al., 2002; Wustman et al., 2001)
Plantago	*Plantago major*	Field	20 yrs	• Population structure • (Davison and Reiling, 1995; • Lyons et al., 1997) • O_3 resistance • (Reiling and Davison, 1992c) • Adaptation (Davison and Reiling, 1995)
Carpathian Mountains	*Pinus sylvestris, Picea abies*	Field	15 yrs	• Significant foliar injury • Community composition changes • Species diversity changes

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

AX9.6.2.3 Sierra Nevada Mountains

Like the San Bernardino Mountains, the western slope of the Sierra Nevada Mountains in central and southern California has also been exposed to elevated O_3 for a long time, although the effects have been much less.. Symptoms of O_3 injury have been found on Ponderosa and Jeffrey pines in all of the Sierra Nevada national forests and parks (Carroll et al., 2003). First identified as a problem in the 1970s (Miller et al., 1972), elevated O_3 with daytime means of 60-80 ppb are common (Bauer et al., 2000; Böhm et al., 1995; Bytnerowicz et al., 2002c; Panek

Table AX9-24. The Most Comprehensively Studied Effects of O_3 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	
• Above-ground	Grulke and Balduman (1999)
• Below-ground	Grulke et al. (1998a); Grulke and Balduman (1999); Grulke and Balduman (1999); Grulke et al. (2001); Arbaugh et al. (1998); Miller and Rechel (1999)
• Root/shoot ratio	
• Carbon allocation	
• Crown vigor	
Ecosystem	
Community dynamics/succession Simulations	Arbaugh et al. (2003); Arbaugh et al. (1999); McBride and Laven (1999)
Understory vegetation	Temple (1999)
Pest interactions	
• Bark beetle/predators	Dahlsten et al. (1997); Pronos et al. (1999)
• Disease occurrence	Miller and Rechel (1999); Pronos et al. (1999)
• Litter decomposition	
Disturbance	
• Bark beetle occurrence	Miller and McBride (1999b); Minnich et al. (1995); Minnich (1999)
• Fire frequency	

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
Foliar Biochemistry and Tissue Chemistry				
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)
Gas Exchange				
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
Growth and Productivity				
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)
Ecosystem Level				
Competitive indices	n.d.	d	i	Miller and Rechel (1999)

[1]Responses are shown as significant increases (i), significant decreases (d), both significant decreases and increases reported (di), nonsignificant effects (n.s.), and no data (n.d.) compared to trees or seedlings at field sites with lower O_3, drought stress, or lack of significant N deposition (<10 kg ha^{-1} yr^{-1}). Frequently n.d. was used for lack of a control site without compounding high N deposition. Foliar analyses and leaf surface properties were largely determined from previous year needles. Gas exchange data were generally from previous year needles at peak growing season, prior to late summer drought (mid- to late July).

[2]Abbreviations: C = carbon; N = nitrogen; A_{max} = maximum photosynthesis rate.

et al., 2002). The west-slope Sierra Nevada forests are also exposed to a wide range of additional gaseous and particulate pollutants, including various S and N compounds (Bytnerowicz et al., 1999; Fenn et al., 2003b; Takemoto et al., 2001), but at levels much lower than in the San Bernardino Mountains. Typical O_3-induced visible foliar symptoms, including chlorotic mottle, chlorophyll degradation, and premature senescence, are commonly found on O_3-sensitive genotypes of Ponderosa pine (Arbaugh et al., 1998; Peterson et al., 1991; Staszak et al., 2004) and Jeffery pine (Arbaugh et al., 1998; Grulke et al., 2003b; Patterson and Rundel, 1995; Peterson et al., 1987). Other important conifers in the region, such as giant sequoia, appear to be relatively O_3-tolerant (Grulke et al., 1996). The symptoms of foliar injury and

growth reductions have been verified on seedlings in O_3 exposure chambers (Momen et al., 2002; Momen and Helms, 1996; Temple, 1988).

Ozone foliar injury of dominant pine species in the Sierra Nevada Mountains is correlated to decreased radial growth in both Ponderosa pine (Peterson et al., 1991) and Jeffrey pine (Patterson and Rundel, 1995; Peterson et al., 1987). Because of the large amount of intraspecific variation in O_3 sensitivity in these two species, O_3 exposure may be a selective agent (Patterson and Rundel, 1995), with differential mortality rates for sensitive individuals (Carroll et al., 2003). The region's forests may also be experiencing subtle changes in species composition and community structure (Patterson and Rundel, 1995; Takemoto et al., 2001).

Based on fire scar dating, reconstructions of stand age classes, historical records, and present stand structure, fire has been largely excluded in western forests for the last 75 to 100 years (Minnich, 1999; Minnich and Padgett, 2003). Fire exclusion has resulted in fewer large stand-replacing fires rather than a mosaic of smaller low-intensity fires. The change in fire intensity may have selectively altered stand structure, fitness and competitiveness of component species, along with their susceptibility to atmospheric pollutants and other stressors (Minnich, 1999). Short-lived (50 to 80 years) species such as knobcone (*Pinus attenuata*) and Coulter pine (*Pinus coulteri*), which occur at the interface of the chaparral and the mixed conifer forest, may already have been selected for O_3 tolerance by seedling establishment (the most sensitive tree age class in conifers) after large fires in the 1950s (Minnich, 1999). Strong measures to suppress fires have largely kept chaparral fires from invading the mixed conifer forests, and stand densification in the mixed conifer zone has increased. High stand density, in turn, may weaken the younger cohorts and increase sensitivity to atmospheric pollution (Minnich, 1999).

Other disturbances that play a potential role in sensitivity to atmospheric pollution include cycles of drought stress. Nearly every decade is marked by one or more years of very low precipitation (Graumlich, 1993). During extended periods of drought, foliar injury is lower than in subsequent years with higher average precipitation (Carroll et al., 2003). In the first several years (1975 to 1977) of a Sierran-wide assessment of O_3 injury to pines, O_3 injury increased, because of greater water availability due to greater stomatal conductance and, presumably, greater O_3 uptake. Trees instrumented with monitors to directly measure canopy transpiration had 20% greater stomatal conductance in mesic microsites (riparian areas, mid-slope seeps) than trees in xeric microsites (rock outcrops) (Grulke et al., 2003a). Although the Sierra Nevada

experienced a prolonged drought between 1987 and 1993, it was less severe than other droughts and O_3 injury did not significantly decrease (Carroll et al., 2003). The same plots showed only a slight increase in O_3 injury between 1993 and 2000. While drought stress may make trees more susceptible to insect and pathogen infestation, serious outbreaks of insect infestation are believed to be indicators, not a cause, of existing stress in the forest (Wickman, 1992).

AX9.6.2.4 Appalachian Mountains

The southern Appalachian Mountain region experiences some of the highest O_3 exposures of any natural areas in the eastern United States (Chappelka et al., 1997; Hildebrand et al., 1996; Mueller, 1994; Samuelson and Kelly, 1997). Since the region is the home of the Shenandoah and Great Smoky Mountains NPs, which have Class I air quality designations by the 1977 Clean Air Act, there has been considerable study of the region's dominant forest species to determine O_3 effects. Visible foliar symptoms of O_3 have been found in natural ecosystems consisting of sassafras (*Sassafras albidum*) (Chappelka et al., 1999b), black cherry (*Prunus serotina*) (Chappelka et al., 1997, 1999a; Hildebrand et al., 1996; Samuelson and Kelly, 1997), yellow poplar and white ash (*Fraxinus americana*) (Chappelka et al., 1999b; Hildebrand et al., 1996). Visible foliar symptoms induced by O_3 have been recreated on the same species in chamber studies (Chappelka et al., 1985; Chappelka and Chevone, 1986; Duchelle et al., 1982; Fredericksen et al., 1995; Samuelson, 1994). No response to O_3 exposure has been found for other hardwood trees, nor for the three conifer species tested (Neufeld et al., 2000).

Long-term foliar injury symptoms have been correlated with decreased radial growth in yellow poplar and black cherry (Somers et al., 1998) and with decreased biomass in cherry (Neufeld et al., 1995). Although climatic conditions (drought) largely explained radial growth reductions, O_3 exposure may have also contributed (McLaughlin and Downing , 1996). Ozone exposure may also be affecting the understory vegetation in the region (Chappelka et al., 1997; 2003; Davison et al., 2003; Duchelle et al., 1983; Duchelle and Skelly, 1981) and community composition (Barbo et al., 1998), through impacts on both growth and reproduction (Chappelka, 2002). Foliar litter from trees exposed to elevated O_3 have lower decomposition rates (Kim et al., 1998). Other air pollutants are likely to be found in this ecosystem but not at the high deposition values found in the California studies. A decline in forest health in the northern Appalachians has been primarily attributed to the effects of acidic fog and rain on soil

acidification, lower Ca^{2+} availability, reduction in fine root biomass, and modification of cuticular wax. However, fog- and O_3-exposed red spruce forests in New England also show winter injury (Percy et al., 1992).

AX9.6.2.5 *Plantago* Studies in the United Kingdom

One of the most well-documented studies of population and community response to O_3 effects are the long-term studies of common plantain (*Plantago major*) in native plant communities in the United Kingdom (Davison and Reiling, 1995; Lyons et al., 1997; Reiling and Davison, 1992c). Sensitive populations of *P. major* had significant growth decreases in elevated O_3 (Pearson et al., 1996; Reiling and Davison, 1992a,b; Whitfield et al., 1997) and reduced fitness as determined by decreased reproductive success (Pearson et al., 1996; Reiling and Davison, 1992a). While spatial comparisons of population responses to O_3 are complicated by other environmental factors, rapid changes in O_3 resistance were imposed by ambient levels and variations in O_3 exposure (Davison and Reiling, 1995). Molecular patterns of genetic variation suggest that a change in O_3 resistance over time probably resulted from natural selection in genotypes already present in local populations, rather than through an influx of new *P. major* germplasm (Wolff et al., 2000). At the site of common plantain seed collection the highest correlations occurred between O_3 resistance and ambient O_3 concentrations occurred (Lyons et al., 1997), rather than between O_3 resistance and other climatic variables, as found for aspen (Berrang et al., 1991).

AX9.6.2.6 Forest Health in the Carpathian Mountains

The Carpathian Mountains cross five countries (the Czech Republic, the Slovak Republic, Poland, Romania, and the Ukraine) and contain many national parks and several biosphere reserves. The forests were largely cleared in the 15th century and were replanted with Norway spruce. As elevation increases, beech (*Fagus sylvatica*) or beech-fir (*Abies alba*) forests grade into Norway spruce or spruce-fir forests. Near the treeline, Norway spruce mixes with dwarf mountain pine (*Pinus mugo*). Dwarf mountain pine forms an almost pure stand just below the alpine vegetation.

The forests of the Carpathian Mountains have been subjected to anthropogenic stressors (e.g., shepherding, metal mining, wood harvest for structures and paper) for hundreds of years,

as described for the Tatra Mountains in the southern Carpathians (Wezyk and Guzik, 2002). The Carpathians have been subjected to regional air pollution stressors since industrialization. Most of the effects of air pollution on forest health degradation were due to (1) heavy metal deposition, (2) soil acidification by acid deposition, and (3) subsequent pest outbreaks, the combination of which led to the forest decline and dieback between 1970 and 1989 (Dunajski, 2002). Industrial pollutants such as SO_2 and heavy metals have significantly declined since the 1980s, but O_3 exposure has continued to increase (Bytnerowicz et al., 2002a; Bytnerowicz et al., 2004). The increased ownership and use of private cars in Central Europe, as well as the long-range transport of O_3 from western Europe, are believed to be responsible for the continued increase in photooxidants. In 1995, drought resulted in significant forest mortality, as well as an epidemic of bark beetle infestation in subsequent years. A network of air quality monitoring sites was installed across Europe in the late 1980s as part of the International Cooperative Programme on Assessment and Monitoring of Air Pollutant Effects on Forests (ICP Forests). Mean defoliation rates for six important forest species across Europe have increased or remained unchanged from 1989 to 1999 (Percy et al., 2002). Ozone concentrations experienced in the Tatra Mountains, especially along the southern slopes, occasionally reach 190 to 200 ppb as 2-week-long averages, with the highest values experienced in early summer at elevations of 1700 to 2300 m (Bytnerowicz et al., 2004). In other parts of the Carpathian Mountains, peak 2-week average O_3 concentrations were lower, at 160 ppb (Bytnerowicz et al., 2002b). For all trees inventoried, about 13% exhibited greater than 25% defoliation during 1997 to 2000. There was no difference between extent of damage for broadleaves or conifers. Trees in Poland and the Czech Republic were the most affected by air pollution, while the least damaged forests were in Romania (Bytnerowicz et al., 2002b).

The extent to which O_3 exposure affects forest health degradation, and slows forest degradation, is still unknown in Europe. In many of the published studies, the response to a known O_3 gradient is largely confounded by other pollutants and/or climatic gradients (Szaro et al., 2002; Widacki et al., 2002). Current levels of ambient O_3 are believed to be high enough to reduce bole radial growth (Percy et al., 2002). Although average O_3 concentration alone was not related to bole growth, the peak hourly O_3 concentration was negatively correlated to bole growth (Muzika et al., 2004). Recent evidence indicates that canopy health of European white

oak (*Quercus robur*), Norway spruce, maritime pine (*Pinus pinaster*), and beech has significantly declined (Huttunen et al., 2001). However, the canopy health of Scots pine has improved. The network of air quality monitoring stations and forest plots is extensive and active. Subsequent correlative analyses including both meteorological and air quality attributes throughout the European Union (EU) will help to determine the specific role of O_3 exposure in forest decline. Historical effects of anthropogenic disturbance may still be confounding.

AX9.6.2.7 Field Exposure System (FACE), Rhinelander, Wisconsin

The Aspen Free-Air CO_2 Enrichment facility was designed to examine the effects of both elevated CO_2 and O_3 on aspen (*Populus tremuloides*), birch (*Betula papyrifera*), and sugar maple in a simple reconstructed plantation characteristic of Great Lakes Aspen-dominated forests (Karnosky et al., 2003b; Karnosky et al., 1999). Instead of using chambers to expose the plants to desired gas concentrations, the gas is piped up vertical delivery tubes in the open air. The vertical delivery pipes surround a 30-m diameter circular plot with five different aspen clones in half of the plot, one quarter of the plot planted in aspen and birch, and one quarter in aspen and maple. The O_3 treatment for the first 5 years was 1.5× ambient, with ambient O_3 exposures averaging 35 to 37 ppb (12 h daytime average over the growing season) compared to elevated O_3 rings averaging 49 to 55 ppb for the same time period (Karnosky et al., 2003b).

Elevated CO_2, elevated O_3, and elevated $CO_2 + O_3$ have had effects on most system components being measured in the study (Table AX9-26) (Karnosky et al., 2003b). One interesting finding of the project has been the nearly complete offset by elevated O_3 of the enhancements induced by elevated atmospheric CO_2 for the pioneer keystone species aspen (Isebrands et al., 2001) and birch (Percy et al., 2002) even though O_3 exposure alone did not always result in a significant response when compared to controls. They also found evidence that the effects on above- and below-ground growth and physiological processes have cascaded through the ecosystem, even affecting microbial communities (Larson et al., 2002; Phillips et al., 2002). This study also confirmed earlier observations of changes in trophic interactions involving keystone tree species, as well as important insect pests and their natural enemies (Table AX9-26) (Awmack et al., 2003; Holton et al., 2003; Percy et al., 2002).

Table AX9-26. Summary of Responses of *Populus tremuloides* to Elevated CO_2 (+200 µmol mol^{-1}), O_3 (1.5 × ambient), or CO_2+O_3 Compared with Control During 3 Years of Treatments at the Aspen FACE Project (Modified from Karnosky et al. (2003b)

	CO_2	O_3	CO_2 + O_3	Reference
Foliar Gene Expression and Biochemistry				
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)
Gas Exchange				
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)
Growth and Productivity				
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)

[1]Responses are shown as significant increases $p < 0.05$ (I), significant increases $p < 0.01$ (ii), significant decreases $p < 0.05$, significant decreases $p < 0.01$ (dd), both significant increases and decreases reported (id), nonsignificant effects (n.s.), and no data (n.d.).

AX9.6.3 Landscape Condition

In the SAB framework (Figure AX9-20), landscape condition is assessed using the areal extent, composition of component landscape ecosystems or habitat types, and the pattern or structure of component ecosystems or habitat types (including biocorridors). To date, no publications exist on the impacts of O_3 exposure on landscape condition. The effects of O_3 exposure have only been reported at the community or stand level (see Biotic Conditions, below). The following is a description of current discussions by land stewards and of how difficult it will be to quantitatively assess the effect of O_3 exposure on landscape condition.

Landscapes are identified and preserved, such as national parks, Class I wilderness areas, etc., so that they are protected from the effects of O_3 exposure by law. Efforts to determine whether landscapes have been affected by certain levels of exposure rely on valuation of landscape and ecosystem components. Several different approaches of valuation have been used, including pathological (visible symptoms), biomass and allocation, and biogeochemical.

In the pathological approach, a "critical levels" concept is developed, with varying levels of impact viewed as acceptable, interim targets, or as unacceptable. As an example, land managers of Class I wilderness areas may consider a level acceptable if it resulted in no visible O_3 symptoms to sensitive species. In concrete terms, sensitive species may respond to peak O_3 exposures of 60 ppb (e.g., coneflower, in Great Smoky National Mountains NP; [Davison et al., 2003]), and so the critical exposure level would be < 60 ppb for any hourly value during the growing season. An interim target would be that less than 5% of the sensitive plants would have visible symptoms on <15% of the leaf surface. An unacceptable level of O_3 exposure would be any result more pronounced than the interim target. The advantage of the foliar injury approach is that large crews with relatively simple training can assess individual species within the landscape and "see" the effect of the oxidant exposure. There are several disadvantages, however. Some species (e.g., white fir) exhibit no foliar injury but do have shifts in biomass allocation in response to oxidant exposure (Retzlaff et al., 2000). Other species have shown significant decreases in foliar injury due to needle loss, with retranslocation of nutrients to remaining foliage, and subsequent increased photosynthetic rate (Beyers et al., 1992). In addition, the development of foliar symptoms within a species is related to sunlight and microclimate (Davison et al., 2003).

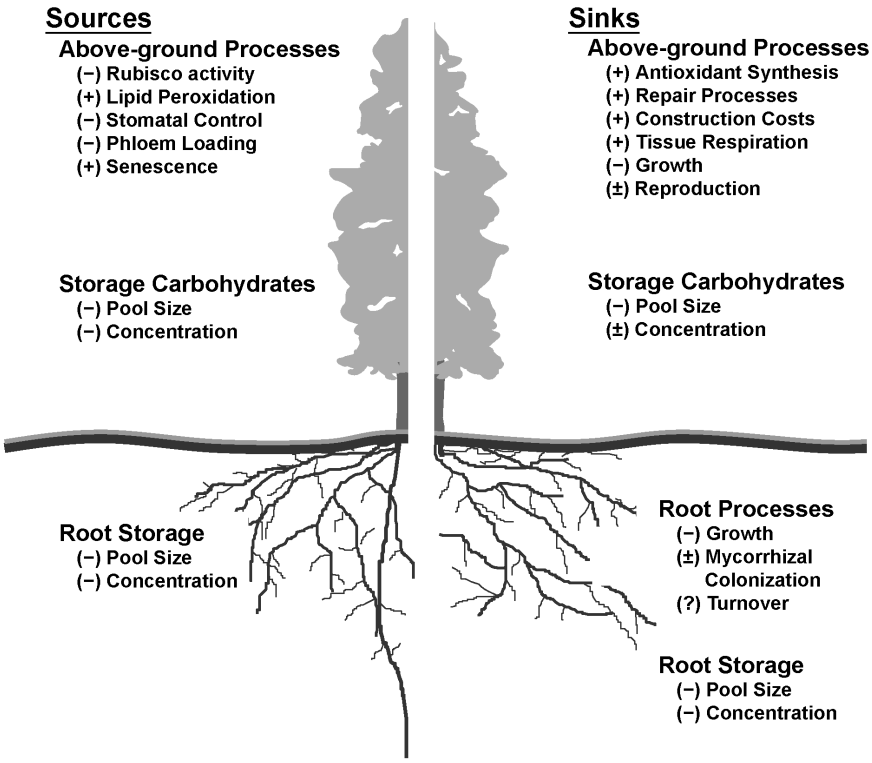


Figure AX9-20. A conceptual diagram of processes and storage pools in sources and sinks that are affected by O_3 exposure. A plus (+) denotes an increase in process rate or pool size, a minus (−) denotes a decrease in process rate or pool size, and a plus-minus (±) denotes that both increases and decreases have been reported in response to O_3. Primary effects in the shoots (1°) are distinguished from secondary effects in roots (2°) since the primary site of O_3 action occurs in the leaves.

Source: Andersen (2003).

In the biomass approach, O_3 exposure resulting in a measurable decline in biomass (usually of a target, sensitive species) is used to evaluate landscape condition. The bulk of the information available is from seedling responses to controlled chamber exposures, reviewed in the previous section. Some information exists for species in natural environments, but teasing out concurrent stressors and finding adequate controls may be intractable. For example, in a long-term gradient of O_3 exposure, N deposition, and drought, the site with the highest O_3 exposure had the greatest whole tree biomass (pole-sized trees) due to growth stimulation by N deposition (Grulke and Balduman, 1999).

In the biogeochemical approach, changes in biogeochemical cycling are used to assess landscape condition. Ozone-sensitive species have well known responses to O_3 exposure, including altered C allocation to below- and above-ground tissues, and altered rates of leaf production, turnover, and decomposition. Changes in turnover rates of ephemeral tissues (leaves, fine roots) also affect nutritional status of the remaining tissue. These shifts can affect overall C and N loss from the ecosystem in terms of respired C, and leached aqueous dissolved organic and inorganic C and N. Instability in C and N pools and their dynamics can affect landscape-level nutrient dynamics even without significant inputs of N deposition. The endpoint assessment is based on changes in water quality from or in the landscape, correlated to a defined oxidant exposure level. These approaches are linkable: visible injury at a particular level could be related to reduction in photosynthate, which would reduce whole plant biomass (and carbon dynamics). If O_3-sensitive species dominate the landscape, then changes in C and N dynamics over time would be expected to alter biogeochemical cycles. Examples of forest types that contain geographically extensive, O_3-sensitive species that could be used in assessing landscape-level changes include Ponderosa pine in the western United States, yellow poplar or loblolly pine in the eastern United States deciduous forests, and Norway spruce in the Carpathian Mountains of eastern Europe.

Water quantity may also be affected by O_3 exposure at the landscape level. Moderately high O_3 exposure may affect the mechanism of stomatal opening (McAinsh et al., 2002), resulting in sluggish stomatal opening and closing (Reich and Lassoie, 1984). During moderately high O_3 exposure in a drought year, canopy transpiration was greater for yellow poplar than on adjacent days with lower O_3 exposure, which could alter water use at the landscape level. Oxidant exposure (O_3 and NO_x) may decrease the ability of exposed plants to close stomata at night (Grulke et al., 2004), thus increasing water loss from the landscape. Ecosystem models should aid in interpreting O_3-exposure effects at the landscape level.

AX9.6.4 Biotic Condition

AX9.6.4.1 Ecosystems and Communities

The SAB framework described by Young and Sanzone (2002) identifies community extent, community composition, trophic structure, community dynamics, and physical structure as EEAs for assessing ecosystem health.

COMMUNITY EXTENT

Ecosystem function is dependent on areal extent, constituent species composition, trophic structure and its dynamics, and community physical structure. Genetic variation within species, and the dynamics of the interactions that exist among different species and their biotic and abiotic environment, are also involved (Agrawal and Agrawal, 2000). There are no reports of O_3 exposure altering community distribution or extent.

COMMUNITY COMPOSITION

Significant changes in plant community composition resulting directly from O_3 exposure has been demonstrated in two forested areas: the mixed conifer forest of the San Bernardino Mountains, CA and the mixed conifer forest of the Valley of Mexico near Mexico City. It is also likely that community composition has changed in response to O_3 exposure in the coniferous forests of the Carpathian Mountains, but this has not yet been definitively shown.

The first forest communities shown to be affected by O_3 were the *Pinus ponderosa*-dominated stands of the San Bernardino Mountains in southern California (Miller, 1973). Miller suggested that mixed forests of *P. ponderosa*, *Pinus jeffreyi*, and *Abies concolor* were changing to predominantly *A. concolor* because of the greater O_3 sensitivity of the pines. Significantly greater mortality of young mature trees (50 to 99 years old) occurred in sites that also showed higher foliar injury relative to sites that showed slight foliar injury (McBride and Laven, 1999). For *P. ponderosa*, 33% of the trees in the high foliar injury sites died versus 7% of the trees in the low foliar injury sites over the decade-long census. In contrast, 24% of *Abies concolor* died in high foliar injury sites, whereas no trees died in slight injury sites. The authors suggested that certain age classes were especially sensitive to O_3 exposure, because they are emerging into the canopy where higher O_3 concentrations are encountered. Future projections based on past changes in community composition have been conducted for 2024 and 2074 (McBride and Laven, 1999). In their projections, the population of Ponderosa pine nearly disappears in all tree age classes, and the community is dominated by California black oak (*Quercus kelloggii*) in all tree age classes, followed by Incense-cedar (*Calocedrus decurrens*) and sugar pine (*Pinus lambertiana*) by the year 2074. Their projections do not account for potential changes in genetic structure of the more O_3-sensitive species.

In the Valley of Mexico, the closed forest structure changed to a woodland from high pollutant exposure (Fiscus et al., 2002). Cryptogamic community diversity also significantly declined in response to prolonged, extreme O_3 exposure (Zambrano and Nash, 2000). Together, these two examples illustrate the potential for shifts in community composition in response to O_3 stress.

TROPHIC STRUCTURE

Above-Ground

One of the first reports of trophic level interactions in natural communities was the O_3-induced predisposition of Ponderosa pine to attack by bark beetles (Cobb et al., 1968; Stark et al., 1968; Stark and Cobb, 1969). Trees exposed to oxidant injury had lower resin production, flow, and exudation pressure. Also, several attributes associated with tree defense against beetle attack including sapwood and phloem moisture content and phloem thickness were compromised by oxidant exposure (Pronos et al., 1999). Another trophic level has been implicated, in that O_3-injured Ponderosa pine had the same rate of bark beetle infection, but healthy trees had greater numbers of bark beetle predators and parasitoids (Dahlsten et al., 1997). This suggests that O_3 damage rendered the pines inhospitable for the natural enemies of the bark beetles. Similar findings were presented by Percy et al. (2002) for aphids whose abundance was increased in young *Populus tremuloides* stands exposed to elevated O_3. In that study, the levels of natural enemies of aphids (ladybirds, lacewings, spiders, and parasitoids) were significantly decreased under elevated O_3.

Below-Ground

Processing of plant-derived carbon compounds by soil organisms comprising the soil food web is a fundamental property of a functional and stable below-ground ecosystem (De Ruiter et al., 1998; Wolters, 1998). Soil food web organisms are responsible for recycling nutrients and for development of soil properties such as porosity, aggregate structure, water-holding capacity, and cation exchange capacity. A shift in food-web species diversity or functional complexity in response to O_3 stress may alter ecosystem processes.

Evidence that soil organisms are affected by O_3 indicates the potential for changes in soil food-web structure and function. Since O_3 does not penetrate the soil beyond a few centimeters,

the proposed mechanism by which O_3 alters soil biota is through a change in C input to soils (Andersen, 2003). Ozone can alter C inputs to soil and hence soil processes through four different pathways: (1) leaf-litter quality and quantity (see Material Cycling, below), (2) C allocation to roots (see Physiological Status, below), (3) interactions among root symbionts, and (4) rhizodeposition. The complex nature of the effects of O_3 on trophic interactions and food webs calls for additional basic research and modeling.

There have been no comprehensive studies on the effects of O_3 on structural components of soil food webs; however, studies have shown that O_3 affects free-living soil organisms of food webs. In the few cases where soil microbial communities have been examined, O_3 has led to changes in bacterial and fungal biomass, and, in some cases, changes in soil enzyme activity. Phillips et al. (2002) examined the effects of elevated CO_2 and O_3 on C flow through heterotrophic microbial communities in soils collected from a FACE study in Wisconsin. Ozone decreased abundance of fungal phospholipid fatty acids in aspen and birch-aspen plots but had few other direct effects on measured soil parameters. The greatest effect of O_3 was to eliminate significant increases in microbial respiration resulting from elevated CO_2, suggesting an important role for O_3 in altering C flow through soils. Shafer (1988) found that O_3 tended to increase the number of fungal propagules and bacteria exhibiting phosphatase activity in the rhizosphere of sorghum. Ozone in combination with simulated acid rain stimulated soil arylsufatase activity (Reddy et al., 1991). The response was observed at low concentrations, but was reversed at high concentrations, suggesting a threshold level of O_3, possibly involving different mechanisms. Ozone significantly decreased soil microbial biomass in the fall after one season of exposure in a wheat and soybean system (Islam et al., 2000). Other studies have shown shifts in microbial and fungal biomass in response to O_3 stress, but responses were variable (Scagel and Andersen, 1997; Yoshida et al., 2001).

Decreased allocation to roots associated with O_3 exposure alters N fixation in legumes and actinorrhizal species. Ozone exposure was found to decrease nodulation in a number of species (Manning et al., 1971; Tingey and Blum, 1973). In alder (*Alnus serrulata*), host root cells of nodules showed cytoplasmic breakdown and lacked organelles when seedlings were exposed to O_3 for 27 days (Greitner and Winner, 1989).

Ozone has been shown to affect mycorrhizal colonization (Adams and O'Neill, 1991; Edwards and Kelly, 1992; Ho and Trappe, 1984; McCool et al., 1982; Simmons and Kelly, 1989;

Smith and Read, 1997). Although short term in nature, several studies have found enhanced mycorrhizal short-root formation under O_3 stress. White pine (*Pinus strobus*) (Stroo et al., 1988), Norway spruce (Rantanen et al., 1994), Northern red oak (*Quercus rubra*) (Reich et al., 1985), Douglas fir (*Pseudotsuga menziesii*) (Gorissen et al., 1991a), European silver fir (*Abies alba*) (Wollmer and Kottke, 1990), and Scots pine (Kasurinen et al., 1999) all showed some increase in mycorrhizal presence when exposed to O_3. Others have shown minimal or no effects of O_3 on mycorrhizas (Kainulainen et al., 2000b; Mahoney et al., 1985; Meier et al., 1990). Stroo et al. (1988) found that percent infection increased from 0.02 to 0.06 ppm O_3, then decreased from 0.06 to 0.14 ppm; the total number of short roots were unaffected, however. In cases where stimulation was observed, the response was often noted shortly after initiation of exposure, often at relatively low concentrations. Good examples of this transitory response can be found in results with Norway spruce and Scots pine (Kasurinen et al., 1999; Rantanen et al., 1994). In these studies, O_3 increased mycorrhizal short roots initially but differences were not evident by the end of the experiment.

Evidence suggests that decreased below-ground allocation associated with O_3 stress alters mycorrhizal host-symbiont compatibility. Edwards and Kelly (1992) found a shift in fungal morphotypes present on loblolly pine roots, even though the number of mycorrhizal short roots per gram fine root was not significantly affected by O_3. Qui et al. (1993) found increased numbers of morphotypes present in O_3-sensitive loblolly pine seedlings exposed to O_3. Roth and Fahey (1998) found an interaction between O_3 and acid precipitation treatments on the composition of fungi forming ectomycorrhizae on red spruce saplings, possibly driven by nutrient availability. Carbohydrate requirements vary among fungal species (Bidartondo et al., 2001), and O_3 may affect species composition by altering carbohydrate availability in roots. A shift in species dominance could lead to a change in successional patterns of mycorrhizal communities.

In the few studies that examined root exudation in response to O_3 exposure, O_3 was found to alter the quantity and quality of root exudates. McCool and Menge (1983) found a significant decrease in exudation of amino acids in tomato exposed to 300 ppb O_3. McCrady and Andersen (2000) observed increased root exudation in nonmycorrhizal wheat. No apparent change in root exudation was found in labeling studies of ECM Ponderosa pine (Andersen and Rygiewicz, 1995a). Inconsistency in the literature probably results from species differences and

experimental protocols; however, these examples illustrate the potential effects of O_3 on rhizosphere C flux.

Decreased C allocation to roots of O_3-exposed plants may reduce root longevity and accelerate root turnover, increasing rhizodeposition of C and N. Fine root turnover decreased in mature northern red oak exposed to elevated O_3 (seasonal exposure ranging from 152 to 189 ppm·h), whereas seedlings did not show any reduction in turnover (Kelting et al., 1995). King et al. (2001) found a trend toward decreased live root biomass and increased dead root biomass in aspen exposed to O_3 in a FACE study, suggesting possible changes in both production and longevity.

Other studies also suggest that O_3 alters C flux to soils, resulting in changes in CO_2 efflux from soils. Both root respiration and soil CO_2 efflux decreased from loblolly pine seedlings exposed to O_3 (Edwards, 1991). Soil CO_2 efflux increased in response to O_3 in Ponderosa pine seedlings (Andersen and Scagel, 1997). No direct assessments of hyphal growth and turnover in response to O_3 stress have been conducted. Ozone decreased C allocation to extrametrical hyphae of a Ponderosa pine mycorrhiza, which might be expected to decrease growth and increase hyphal turnover (Andersen and Rygiewicz, 1995b).

COMMUNITY DYNAMICS, PHYSICAL STRUCTURE

One of the best-documented examples of change in long-term forest community dynamics, in dominant overstory trees, occurred in the San Bernardino Mountains between 1968 and 1974 (as reported by Miller [1973] and Miller et al. [1989]). Plots were recently re-inventoried ~25 years after establishment (Arbaugh et al., 2003). Of the six codominant canopy species, white fir showed the greatest change, increasing in both numbers and bole growth for a 286% change in basal area/ha in the San Bernardino Mountains. Sugar pine basal area also increased significantly (by 334%), but this species represents only a small portion (1%) of the total basal area of the forest sampled. The most O_3-sensitive species (Miller et al., 1982), Ponderosa and Jeffrey pine, had the lowest increase in basal area/ha (76 and 62%, respectively). These two species represented 72% of the basal area/ha of all stands inventoried. Ponderosa pine had the greatest mortality rate of all canopy species inventoried (46%), followed by white fir and black oak (35% and 33%), Jeffrey pine (29%) and Incense cedar and sugar pine (both 7%). In moist sites (at the western end of the San Bernardino Mountains), there was significant recruitment of

Incense cedar, white fir, and sugar pine. Only one study directly attributed tree mortality to O_3 exposure: it accounted for 7% of mortality in the Sierra and Sequoia NFs (Carroll et al., 2003).

Species diversity in the understory can be quite large, making studies of O_3 effects on understory community dynamics very challenging. However, there have been some attempts to quantify understory responses, ranging from describing relative sensitivity to their visible symptoms (Temple, 1999; Treshow and Stewart, 1973) to very complex measures of community structure and composition (Westman, 1979, 1981). The lowest percentage cover and lowest species diversity in California coastal sage scrub was correlated with the highest O_3 exposures as estimated by extrapolation from the closest air monitoring stations (Westman, 1979). The understory also has the potential to influence responses to O_3 of dominant keystone species, as has been shown in controlled experiments with both Ponderosa pine (Andersen et al., 2001) and Loblloly pine (Barbo et al., 2002). Barbo et al. (1998) exposed an early successional forest community to ambient air, charcoal-filtered air, non-filtered air, and 2× ambient in the Shenandoah NP. They found changes in species performance, canopy structure, species richness, and diversity index consistent with the view that O_3 can induce a shift in vegetation dominance and community structure.

There have been few studies evaluating the effect of O_3 exposure on the physical structure of natural ecosystems. Despite an extensive array of allometric equations for conifers in the western United States (Ter-Mikaelian and Korzukhin, 1997), none appear to predict individual tree shape in a site of moderate O_3 exposure, suggesting that O_3 may effect allometry (Grulke et al., 2003a). Canopy structural changes are also implied by the measure of canopy transparency used in the USDA Forest Service's Forest Health Monitoring (FHM) assessment. The loss of epiphytic lichens within the canopy is a clear example of plant community structural change occurring along an O_3 gradient (Nash and Sigal, 1999; Zambrano and Nash, 2000).

As of yet, there have been no comprehensive studies on the effects of O_3 on structural or functional below-ground components (Andersen, 2003). Phillips et al. (2002) found evidence for changes in the bacterial and fungal biomass below aspen and aspen/paper birch stands exposed to elevated O_3. Subsequent study showed that O_3 exposure decreased cellobiohydrolase activity in the soil microorganisms, driving the change in the microbial community (Larson et al., 2002).

AX9.6.4.2 Species and Populations

Ozone can affect species and populations of species found in ecosystems through changes in population size, genetic diversity, population structure and/or dynamics, and habitat suitability (Young and Sanzone, 2002). For example, if individuals of a species are lost due to O_3 exposure, population size declines. Often very young (e.g., conifer seedlings, see Section AX9.6.4.3 below) and old individuals differ in their sensitivity, so that population structure also will be altered by O_3 exposure. If resource allocation to reproductive output is altered by O_3 exposure, population dynamics will be altered. Communities dominated by O_3 sensitive species in the canopy or understory may be altered sufficiently for the habitat to become unsuitable for other species. Genetic selection acts on the individual plant, which represents a certain proportion of the populations' genetic variation. If an O_3-sensitive individual succumbs through multiple stresses, including O_3 stress, the genetic variation represented in the population generally declines, unless sensitive individuals have low inherent genetic variability (e.g., Staszak et al., 2004).

While the concept of natural selection induced by O_3 exposure and related changes in natural plant communities has been around for a long time (Dunn, 1959; Miller et al., 1972), the concept of evolution of O_3 tolerance is still not widely accepted. The unequivocal demonstration that considerable genetic variation in O_3 resistance exists within and between plant populations, and that ambient levels of O_3 may differentially affect fitness-related traits (i.e., growth, survival, and fecundity), suggests that O_3 may potentially drive the natural selection for resistant individuals. Dunn (1959) presented circumstantial evidence that ambient O_3 in the Los Angeles area was high enough to drive the selection of O_3-resistant *Lupinus bicolor* genotypes. Since Dunn's (Dunn, 1959) research on O_3-induced population changes, researchers have demonstrated differences in O_3 tolerance among other plant populations. In the devastating forest decline southeast of Mexico City, the remaining trees (primarily *Abies religiosa* in the "cemetery forests") appear to be less affected by foliar injury than trees that were lost, despite continued high-O_3 exposures (Alvarado et al., 1993). However, even the most convincing work in this field (Berrang et al., 1986, 1989, 1991), with *Populus tremuloides*, where a strong correlation between visible foliar injury after O_3 exposure and maximum O_3 concentration at the origin of the population was shown (Berrang et al., 1991), a change in gene frequency at any one site over time has not yet been demonstrated (Bell et al., 1991; Reiling and Davison, 1992a).

Furthermore, the selection intensity of O_3 has been questioned (Bell et al., 1991; Taylor et al., 1994; Taylor and Pitelka, 1992) and the emergence of O_3 exposure since the 1950s as an environmental stressor may not have been long enough to affect tree populations with long generation times (Barrett and Bush, 1991).

The loss of O_3-sensitive individuals results in natural selection favoring O_3-tolerant species (Bradshaw and McNeilly, 1991). Increased levels of mortality of O_3-sensitive individuals have occurred for *Pinus jeffreyi* and *P. ponderosa* exposed to ambient O_3 along the western slope of the Sierra Nevadas (Miller et al., 1998; Peterson et al., 1987), for *Pinus strobus* exposed to ambient O_3 in southern Wisconsin (Karnosky, 1981), and for *P. ponderosa* in the San Bernardino Mountains (Carroll et al., 2003). In these examples, individuals that consistently had greater foliar injury and lower needle retention were lost in repeated surveys. Ozone-induced loss of all individuals except the most tolerant and breeding among the surviving individuals to yield more more tolerant populations has not yet been demonstrated for plants exposed to O_3, except for the relatively short-term (2 years) adaptation exhibited in *Trifolium repens* (Heagle et al., 1991) and *Plantago major* (Davison and Reiling, 1995). Heagle et al. (1991) were able to show the adaptation of a *Trifolium repens* population to elevated O_3 in just two growing seasons. Similarly, Davison and Reiling (1995) compared the O_3 resistance of *P. major* populations grown from seed collected from the same sites over a period of increasing O_3. The two independent populations studied exhibited increased O_3 resistance, consistent with the idea of selection for O_3 tolerance. Using random amplified polymorphic DNA primers, this team also showed that the later populations are subsets of the earlier ones, consistent with in-situ evolution rather than with catastrophic loss and replacement of the populations (Wolff et al., 2000). The problem is determining whether spatial patterns in O_3 resistance and changes in time are casually related to O_3, because there were very strong correlations with other factors (Davison and Barnes, 1998; Reiling and Davison, 1992c). The potential for the evolution of O_3 resistance has been clearly demonstrated by Whitfield et al. (1997) in their study of O_3 selection of common plantain, where they showed that within a matter of a few generations, it was possible to increase O_3 resistance in an initially O_3-sensitive population. Wild radish (*Raphanus sativus*) developed O_3 resistance after only one generation of exposure to O_3 (Gillespie and Winner, 1989).

A third independent line of research suggesting O_3 may be affecting the genetic diversity of wild plant populations was presented by Paludan-Müller et al. (1999) who showed that northwest European provenances of European beech were more sensitive to O_3 than were southeast European provenances that had experienced higher O_3 levels. Recent research on the genetic structure of 50-year-old Ponderosa pines in the San Bernardino Mountains suggests that distinct differences in frequency of some alleles and genotypes occurred, with the O_3-tolerant trees being more heterozygous (Staszak et al., 2004). While both of these studies were only correlational, they are consistent with previous studies of this type, suggesting O_3-induced population changes. Again, other environmental stressors besides O_3 exposure could have been involved in effecting change within these populations.

Natural selection for O_3 tolerance can also be facilitated by reductions in fitness related to lower seed yields of O_3-sensitive species or individuals. The impacts of O_3 on reproductive development, recently reviewed by Black et al. (2000) can occur by influencing (1) age of flowering, particularly in long-lived trees that often have long juvenile periods of early growth without flower and seed production; (2) flower bud initiation and development; (3) pollen germination and pollen tube growth; and (4) seed, fruit, or cone yields and seed quality (Table AX9-24). In addition, vegetatively propagated species can have lower numbers of propagules under elevated O_3 conditions (Table AX9-24).

Several studies suggest that reproductive structures are clearly sensitive to O_3 and that O_3 can affect fitness of plants by affecting either the sporophytic or gametic generations. Decreased numbers of flower spikes and seed capsules per plant were found for plantain growing under elevated O_3 (Lyons and Barnes, 1998; Pearson et al., 1996; Reiling and Davison, 1992a). Similar responses were seen for *Brassica campestris* plants exposed to a single dose of 100 ppb O_3 for 6 h. Stewart et al. (1996) and Bosac et al. (1998) reported an increase of flower bud abortion for oilseed rape (*Brassica napus* L.) similarly exposed to a short duration of elevated O_3. Floral initiation period can be delayed in O_3-sensitive plants, as was described for dogbane (*Apocynum androsaemifolium*) grown under ambient O_3 in the eastern United States. In one of the few comparisons of whole plant O_3 sensitivities with that of male gametophytes, Hormaza et al. (1996) found a high correlation of relative O_3 sensitivity of pollen tube elongation with that of O_3 effects on net photosynthesis and relative growth rates for 6 species of fruit trees.

Clearly, the concept of O_3-induced genetic change is an area that needs additional research attention. Repeated collections over time from wild populations receiving high O_3 exposures to examine population responses and relative sensitivity changes, the sampling of genetic diversity along known O_3 gradients, and the use of modern biotechnological approaches to characterize and quantify genetic diversity are useful approaches to test for O_3-induced impacts on diversity in natural ecosystems.

AX9.6.5 Organism Condition

PHYSIOLOGICAL STATUS

The generalized effects of O_3 exposure on plants are well known, and have been reviewed from several viewpoints over the last decade (Darrall, 1989; De Kok and Tausz, 2001; Heath and Taylor, 1997; Matyssek et al., 1995; Pell et al., 1997; Reich, 1987; Schraudner et al., 1997; U.S. Environmental Protection Agency, 1996). The topic of individual species response and modification of response by other factors has been addressed thoroughly in Sections AX9.6.3 and AX9.6.4 of this chapter. Here, this subsection will describe the physiological changes in response to O_3 that have been hypothesized to lead to changes in ecosystem structure or function.

Above-Ground Responses

The first critical step leading to O_3 response is uptake of O_3 by the leaves, leading to changes in C and nutrient relations that are thought to alter plant growth and competitiveness (see Section AX9.3). Ozone enters leaves through stomata, reacts with cell walls or membranes, and starts a series of adverse reactions. Cuticular uptake of O_3 is believed to be negligible (Coe et al., 1995; Kerstiens and Lendzian, 1989). Once inside the leaf, O_3 and its byproducts lead to membrane disruption, chlorophyll breakdown, and decreased Rubisco levels (Schweizer and Arndt, 1990). In turn, photosynthesis is decreased, as is stomatal conductance (Weber et al., 1993). Also, O_3 often leads to increased maintenance respiration, decreased foliar nutrient content, and imbalances in tissue nutrient content and retention. When photosynthetic pigments have been damaged, the pigment must be fully broken down (and/or new N and Mg must be taken up and transported to the leaf) for the pigment to be regenerated (Bjorkman and Demmig-Adams, 1995). Ozone exposure alters within-plant priorities for resources: less C is available for allocation to roots and spring regrowth, and less foliar biomass is retained. At the whole-

organism level, O_3 exposure decreases root mass (Grulke et al., 1998a) and radial bole growth (Muzika et al., 2004; Peterson et al., 1991) with little impact on height growth. Visible symptoms of O_3 injury vary between species and genotypes but often include upper leaf surface stipple, chlorotic mottle, or large bifacial blotches of necrotic tissue. Premature senescence is typical of almost all O_3-induced foliar damage. All of these changes can alter the plant's ability to function in a broader ecosystem context.

The underlying mechanisms of O_3-injury response in conifers, broadleaf deciduous trees, and herbaceous species are assumed to be similar. However, several differences in long-lived species are important at the ecosystem level. Most of the research on O_3 effects has been conducted on herbaceous species (i.e., crops). Although a number of native herbaceous species have been identified as O_3-sensitive, there are no published physiological studies on the effect of O_3 exposure on herbaceous or shrub species in situ. In natural ecosystems, the majority of species are not annuals, unless the system is highly disturbed. Nonetheless, response of crop species to elevated O_3 may be used as an analog for native annual response: phenological staging is accelerated (soybeans; Booker et al. [2004]), thus "avoiding" additional O_3 exposure.

Conifers have roughly half the stomatal conductance of deciduous broadleaf trees (Reich, 1987), leading to proportionally less O_3 uptake at the same O_3 exposure level. Yet, except for species of larch, individual conifer needles are longer-lived and active over a greater portion of the year. Therefore, needle longevity can also work against the tree by increasing cumulative O_3 exposure and exposure to other stressors. Increased needle longevity is not always a disadvantage, for example, conifers are physiologically active in early spring and late fall, during times of lower oxidant concentrations. These periods can contribute significantly to a net positive annual C balance and from the standpoint of nutrient storage, are important in reparation responses to pollutants. Patterson and Rundel (1995) reported that Jeffrey pine had significant stomatal opening (one third that of a typical summer day) in midwinter with snow on the ground. At least pole-sized and larger trees can mitigate reductions in C acquisition due to oxidant exposure in the summer with C assimilation on favorable days in the winter. The interaction of environmental factors, plant phenology (the timing of growth events; birth and mortality of plant parts), physiological status (nutritional or moisture status; dormant or active growth within the year), and tree age (interannual differences in resource acquisition and allocation) all contribute to the complexity of long-lived species (and hence ecosystem) response to O_3 exposure.

One widely observed response to O_3 exposure is premature leaf loss. As noted above, premature leaf loss may reduce O_3 uptake during high-O_3 years, but it has several negative consequences. Early leaf loss results in reduced C uptake through photosynthesis. Premature needle loss also results in less N retranslocation compared to normally senescing leaves, which reduces whole plant N balance (Fenn and Dunn, 1989) and carbohydrate availability for overwinter storage (Grulke et al., 2001). Because of such effects accumulated over several years of O_3 exposure, subsequent-year C and N reserves can be affected (Andersen et al., 1991).

Conversely, a series of drought years can decrease O_3 uptake, as well as reduce C and nutrient acquisition, altering resource allocation to defenses (e.g., antioxidants) (Grulke et al., 2003b) (or resins) against insect infestation, rendering the tree more susceptible to O_3 injury. Conifers have thicker cuticles than either broadleaf deciduous or herbaceous species. Continued O_3 exposure may compromise cuticular integrity (Bytnerowicz and Turunen, 1994). Once cuticular integrity is breached, individual leaves (needles) are likely to be excised, thus contributing generally to defoliation and reduced C acquisition.

With the exception of the extensive research conducted on mature tree response to O_3 exposure in California forests (Arbaugh et al., 1998; Grulke et al., 1996, 1998b, 2001, 2002b, 2003a,b, 2004; Grulke, 1999; Grulke and Balduman, 1999; Peterson et al., 1987, 1991, 1995; Wieser et al., 2002), the vast majority of studies of O_3 effects on forest trees have been conducted on young seedlings (Chappelka and Samuelson, 1998) and little is known about acclimation to O_3 (Skärby et al., 1998). Chamber exposure studies can be used to document foliar symptoms and develop response variables for the whole plant. These response variables can then be field tested on mature trees using correlative analyses (e.g., Grulke and Lee [1997]; Grulke et al. [2003b]). Without the initial work in chamber exposure studies, field responses to O_3 exposure would be difficult to verify and distinguish from other concurrent stressors.

Predicting mature tree responses to O_3 solely from seedling response studies is complex, because seedlings or saplings do not necessarily respond to O_3 in the same way as mature trees (Karnosky et al., 2003a; Norby et al., 1999). Although species dependent, O_3 has been found to have stronger effects on leaf function in younger rather than older trees (Kolb and Matyssek, 2001). Each component physiological attribute "matures" at a different rate. Gas exchange patterns differ between seedlings and mature trees. For example, leaf respiration of juvenile Ponderosa pine was greater than that of mature trees (Momen et al., 1996). In the conifers

tested, the highest gas exchange rates (and by inference stomatal uptake of O_3) are found in seedlings (e.g., in scions of red spruce, Rebbeck et al. [1993]; giant sequoia, Grulke and Miller [1994]; Ponderosa pine, Grulke and Retzlaff [2001]; and Norway spruce, Wieser et al. [2002]). Patterns of biomass (Grulke and Balduman, 1999) and carbohydrate allocation (Grulke et al., 2001) differ between immature and mature trees. Pole-sized trees had greater reduction in root, foliar, and bole carbohydrate concentrations than did old growth trees. Antioxidant defenses vary with both tree age and needle age (Tegischer et al., 2002). Based on all attributes measured in both Ponderosa pine and giant sequoia, the youngest tree age considered representative of mature trees was 20 years old (Grulke et al., 1996; Grulke and Retzlaff, 2001). In some broadleaf deciduous tree species, seedlings are more conservative, and mature trees have greater gas exchange rates, as is the case for *Quercus rubra* (Edwards et al., 1994; Kelting et al., 1995; Samuelson and Kelly, 1996, 1997) and *Fagus sylvatica* (Braun et al., 1999). In another broadleaf deciduous tree species (black cherry), gas-exchange rates of seedlings were faster, but total O_3 flux to leaves of seedlings was lower than that of mature trees due to differences in leaf ontogeny (Fredericksen et al., 1995, 1996b).

Nitrogen deposition modifies the effects of oxidant exposure through several offsetting physiological mechanisms (see Section AX9.4.4). Nitrogen deposition, in wet or dry particulate form, ultimately increases site fertility, but increased soil N availability decreases C allocation to roots, further exacerbating the effects of O_3 exposure on roots (Grulke et al., 1998a). Increased N availability also increases foliage turnover: fewer needle age classes are retained (Gower et al., 1993). Therefore, the combination of both increased N and O_3 exposure increases foliar turnover. Finally, N deposition and increased plant N nutrition can increase stomatal conductance, leading to increased O_3 uptake. Alternatively, increased N counteracts the effect of O_3 on photosynthesis by increasing photosynthetic pigments and enzymes. Nitrogen deposition may mitigate the degree of foliar injury from oxidant pollution via higher available N for reparation of photosynthetic pigments. Nitrogen amendments also modify the antioxidant defense system in complex ways (Polle, 1998).

Attributes of O_3 injury to trees (foliar injury, needle retention, and canopy transparency), as well as presence of pathogens and insect infestation, are routinely inventoried in established sample plots distributed on Federal lands across the United States (Forest Health Protection, USDA Forest Service). Foliar injury to several widespread, herbaceous species nationally

recognized as sensitive (bioindicators) is also assessed (U.S. National Park Service, 2003). This assessment is part of a larger assessment of forest tree growth and dynamics (the Forest Inventory and Analysis Program) (Smith et al., 2003; Smith, 2002)). Risk of O_3 injury is then estimated for the dominant forest tree species in the sample plots. For example, 12% of sampled black cherry, 15% of loblolly pine, and 24% of sweetgum (*Liquidambar styraciflua*) were found to be in the highest risk category in the northeast and mid-Atlantic states (Coulston et al., 2003). In the Carpathian Mountains, 12 to 13% of all trees (broadleaf and coniferous) have greater than 26% crown defoliation (Bytnerowicz et al., 2002b). In general, broadleaves (primarily beech) trees were less affected (8 to 45%) than spruce (up to 37%) and fir (up to 50%) (Bytnerowicz et al., 2002b). Ozone injury was directly correlated with cumulative O_3 exposure in the Sierra Nevada Mountains (Arbaugh et al., 1998); with the best correlation being found across sites where >90% of the trees had O_3 injury. Although direct links of visible foliar symptoms induced by O_3 to adverse effects on biomass are not always found, visible foliar symptoms have been linked to decreased vegetative growth (Karnosky et al., 1996; Peterson et al., 1987; Somers et al., 1998), as well as reproductive function (Black et al., 2000; Chappelka, 2002).

Foliar O_3 injury has also been associated with adverse effects on competitive ability and survival in forest communities (Karnosky, 1981; Karnosky et al., 2003a; McDonald et al., 2002). Competition can alter organism condition and affect susceptibility to O_3. Ponderosa pine seedlings were more susceptible to O_3, as determined by decreased plant biomass, when grown in competition with blue wild-rye grass (Andersen et al., 2001). Similarly, the magnitude of O_3 effects on height and diameter growth depended on the competitive status of *Populus tremuloides* trees (McDonald et al., 2002). These studies show the importance of including competition as a concurrent stressor in assessing whole plant responses to O_3. The age of the community ("time since disturbance") may also affect the ability of individuals to effectively respond to O_3. Unfortunately, the vast majority of O_3 studies have been conducted on open-grown plants, often grown in pots where competition is absent both above and below ground.

Clearly, age-dependent O_3 responsiveness and juvenile-mature correlations remain important research questions in attempting to scale up to ecosystem level responses. Patterns of allocation between root, stem, and leaf differ between immature and mature trees. Tree architecture varies with tree age, and leaf area distribution in space and time may change in

response to elevated O_3. All of these factors influence gas exchange in the canopy. Furthermore, there may be few generalities that can be made from seedling to mature tree response to O_3 within a plant functional group (Karnosky, 2003a; Norby et al., 1999). Consequently, modeling ecosystem response is limited to either dealing with monospecific plantations or assigning average responses to a mix of species.

Below-Ground Responses

The effect of O_3 on the soil ecosystem is thought to occur through physiological changes in the root and interactions with soil organisms (Andersen, 2003). Comparatively little is known about how changes in root growth and metabolism are translated through the soil food web, resulting in changes in soil and, hence, ecosystem processes. An overview of physiological changes likely to lead to changes at the ecosystem level is provided below.

Ozone stress decreases C allocation to roots (Cooley and Manning, 1987; Gorissen et al., 1994; Gorissen and Van Veen, 1988; Manning et al., 1971; McCool and Menge, 1983; McLaughlin and McConathy, 1983; Rennenberg et al., 1996; Spence et al., 1990; U.S. Environmental Protection Agency, 1996). Since roots are often dependent on current photosynthate for their structural development (Marshall and Waring, 1985; Ritchie and Dunlap, 1980; Van Den Driessche, 1978, 1991), C-limiting stressor such as O_3 can have rapid and significant effects on root growth. In many cases, decreased allocation to roots in response to O_3 occurs quickly, with reductions in root growth occurring within one growing season (Andersen and Rygiewicz, 1995a; Andersen and Rygiewicz, 1991; Gorissen et al., 1991b; Gorissen and Van Veen, 1988; Spence et al., 1990; U.S. Environmental Protection Agency, 1996). Decreased C allocation below ground is often associated with decreased root-shoot ratio, but observed responses in root-shoot ratio are highly variable owing to several factors including intra- and interspecies variation, culture conditions, and ontogenetic drift (Reich, 2002). Root-shoot ratio is a point-in-time measurement that does not include C lost to exudation, respiration, or turnover. Therefore, biomass and ratios of biomass (such as root-shoot ratio) do not necessarily reveal physiological changes in response to O_3 stress.

Decreased C acquisition leads to reduced carbohydrate levels and storage pools in O_3-exposed plants (Andersen and Scagel, 1997; Cooley and Manning, 1987; Gorissen et al., 1994; Ito et al., 1985; McLaughlin et al., 1982; Rebbeck et al., 1988; Tingey et al., 1976b).

Although it is difficult to quantify changes in the field, Grulke et al. (1998a) found decreased medium and fine root biomass with increased pollutant load across an O_3 gradient in southern California. Coarse and fine root starch concentrations also were lowest in mature trees at the most polluted site (Grulke et al., 2001). The effects of O_3 could not be completely separated from other known stresses across the pollutant gradient, but it appeared that O_3 was an important factor in the patterns observed.

Decreased storage pools can lead to carry-over effects on root growth that are compounded over time. Decreased carbohydrate storage pools were associated with decreased root growth during the spring following exposure to O_3, even in the absence of additional O_3 exposure (Andersen et al., 1991, 1997). Decreased spring root growth was attributed to decreased stored C reserves as well as to premature loss of older foliage age classes during the previous fall. Aside from the loss of photosynthetic surface area associated with premature senescence, early loss of foliage in the fall occurs when allocation to roots is at a maximum in many species (Kozlowski and Pallardy, 1997). Older needle age classes preferentially allocate photosynthate basipetally to stems and roots (Gordon and Larson, 1970; Rangnekar et al., 1969); and the loss of older needles in the fall during allocation to root growth and storage; and in the spring during periods of root growth, preferentially impacts roots and root processes.

Ozone has also been shown to affect root metabolism as evidenced by changes in root respiration. Edwards (1991) found decreased root and soil CO_2 efflux during a 2-year exposure of loblolly pine to O_3. Fine root respiration increased in mature red oak exposed to O_3, while total soil CO_2 efflux increased in the spring and decreased in the summer and fall (Kelting et al., 1995). The authors attributed increased root respiration to increased nutrient uptake in support of increased demands in the shoot. Ozone decreased root system respiration in aspen after 12 weeks of exposure, but the decrease was closely associated with decreased root biomass and probably not metabolic processes (Coleman et al., 1996). Whether other metabolic shifts occur in the roots of plants exposed to O_3 needs to be examined.

Measurable effects on roots may occur before effects on shoots are observed because shoots have immediate access to C for repair and compensation, whereas roots must compete with shoots for C. Mortensen (1998) found decreased root, but not shoot, growth in *Betula pubescens* at O_3 exposures of 42 nMol mol-L (applied 12 h day^{-1}), whereas both root and shoot growth were reduced at higher exposures. Chromosomal aberrations were found in root tips of

Norway spruce exposed to O_3, even in the absence of biochemical changes in needles (Wonisch et al., 1998, 1999). Using relatively high O_3 concentrations (0.15 ppm O_3 6 h day^{-1}), Hofstra et al. (1981) found metabolic changes in *Phaseolus vulgaris* root tips prior to the development of leaf injury. Morphological changes in root tips occurred within 2 to 3 days, and metabolism declined within 4 to 5 days of initiation of O_3 exposure.

Feedback signals from roots can influence the degree of O_3 response. Stolzy et al. (1964) exposed tomato roots to periods of anaerobic conditions and tracked a change in leaf susceptibility to O_3. An exposure of roots to low oxygen conditions for 3 h did not alter photosynthesis, but foliar damage was decreased when the roots were subsequently exposed to O_3. In this case, a signal originating in the root appeared to alter leaf sensitivity to O_3, the signal possibly being hydraulic in nature and leading to decreased O_3 uptake.

SYMPTOMS OF DISEASE OR TRAUMA, SIGNS OF DISEASE

Although insects and diseases are dynamic components of forest ecosystems, trees can be especially susceptible to outbreaks due to the presence of multiple stressors such as drought and pollutant exposure. Ozone can have direct effects on insect or disease organisms, indirect effects on the insect or pathogen through changes to the host, and direct or indirect effects on natural enemies of the insect or pathogen (Pronos et al., 1999). A full discussion of O_3 effects on insect and pathogen interactions can be found in Section AX9.4.

Although the multitude of interacting factors makes it difficult to identify causative factors in the field, some recent examples suggest a role for O_3 in the timing or magnitude of disease attacks in the field. After periods of drought stress (such as 1995 in central Europe), the incidence of bark beetle (*Ips* spp.) appears to increase. In 1998 and 1999, the mean daily capture of *Ips* was lowest in plots with low O_3 exposure; the converse was also true (Grodzki et al., 2004). Elevation confounded the relationships, but the differences in *Ips* frequency in relation to O_3 concentrations were highly significant at lower elevations. In the Valley of Mexico, a 1982 to 1983 drought was documented, but not described as precipitating a bark beetle attack in the early 1980s. However, a link between air pollutant exposure and bark beetle attacks were implicated, because attacked trees were already O_3-stressed at the time of the bark beetle attack (Alvarado et al., 1993).

An early study showed that oxidant exposure predisposed Ponderosa pine to the root pathogen *Fomes annosus* (James et al., 1980). Both root diseases (Pronos et al., 1999) and O_3 exposure (Grulke and Balduman, 1999) can each reduce root biomass, leading to increased drought stress, insect attack, and subsequent windthrow or death. Trees may take several years to die, and the patterns of precipitation and annual total rainfall interact to drive the level of drought stress experienced by the tree (Pronos et al., 1999). Additional research is necessary to fully understand the complex interactions occurring between O_3 stress and other biotic stresses.

AX9.6.6 Ecosystem, Chemical, and Physical Characteristics (water, soil)

AX9.6.6.1 Nutrient Concentrations, Trace Inorganic, and Organic Chemicals

Ozone exposure reduces the nutritional content of tissues, as well as causing elemental imbalances. Foliar nutrient content may be too high (toxic) or too low (deficient), but the relative amounts and ratios among all nutrients can also result in imbalances.

Although N deposition and foliar N content increased with O_3 exposure in the San Bernardino Mountains, K, Mg, Fe, and Al were all also higher in Ponderosa pine at sites more exposed to air pollution (Poth and Fenn, 1998). Trees with greater foliar injury (due to O_3 exposure) had higher current-year needle concentrations of P, K, Zn, and Fe than trees at the same site that were less injured. In drought-stressed Ponderosa pine with O_3 exposure, foliar N was also elevated and retained in the remaining needles (Temple et al., 1992). At a relatively clean site in the eastern San Bernardino Mountains, N, P, and K were efficiently readsorbed, but the amount of P remaining in the foliage was relatively high compared to defined thresholds. The fact that other elements were modified besides the N being deposited emphasizes the degree of chemical imbalance in the tissue. Foliar micronutrients were within the normal ranges reported for Ponderosa pine (Poth and Fenn, 1998; Powers, 1981). Because both N deposition and O_3 exposure reduce root biomass, it was unlikely that the foliar nutrient content was higher due to greater uptake. Instead, it appears that retranslocation from senescing tissue was responsible. Across a pollution gradient in the Carpathian Mountains in eastern Europe, only S/N (expected due to high S deposition) and Fe/Mn ratios were out of balance relative to established norms. The S was relatively high due to SO_2 deposition, and the Fe was relatively high due to smelter plumes. No imbalances could be directly attributed to O_3 exposure (Mankovska et al., 2004).

AX9.6.7 Ecological Processes

AX9.6.7.1 Energy Flow

All green plants generate and use energy-containing C compounds through the processes of photosynthesis and respiration. Whole-plant C uptake is dependent on photosynthesis rates, leaf area, and leaf phenology. The effects of O_3 at the site of action in the leaf are discussed in Section AX9.2. Here, the main focus is on whole-plant carbon dynamics resulting from changes in C acquisition or use under O_3 stress.

In natural ecosystems, O_3 has been shown to depress photosynthesis in sensitive tree species including Ponderosa pine (Grulke et al., 2002b; Miller et al., 1969; Takemoto et al., 1997; Weber et al., 1993) and aspen (Coleman et al., 1995a; Noormets et al., 2001a,b; Sharma et al., 2003; Yun and Laurence, 1999a). In a study of mature Jeffrey pine, trees in mesic microsites had greater O_3 uptake over the growing season in comparison to trees in xeric microsites (Grulke et al., 2003a) and greater O_3 uptake was correlated with lower mid-canopy needle retention, lower branch diameters, and lower foliar N content (Grulke et al., 2003a). Chamber studies have also shown negative effects of O_3 on tree seedling canopy structure (Dickson et al., 2001) and leaf area (Neufeld et al., 1995; Wiltshire et al., 1994). It is well known from chamber and field studies that O_3 exposure is correlated with lower foliar retention (Grulke and Lee, 1997; Karnosky et al., 1996; Miller et al., 1963, 1972; Pell et al., 1999; Topa et al., 2001).

In contrast to the relatively consistent findings for photosynthesis, O_3 effects on respiration have been more variable. Stem respiration was unaffected by O_3 exposure (Matyssek et al., 2002), suggesting that construction costs of new stems are not affected by O_3. However, the bole represents a relatively large storage pool of carbohydrates in mature trees, and the timing of phenological events among individual trees may help to confound the ability to statistically detect differences in stem respiration across pollutant gradients (Grulke et al., 2001). Below-ground respiration has been found to both increase and decrease in response to O_3, depending on the approach and timing of CO_2 measures (Andersen and Scagel, 1997; Coleman et al., 1996; King et al., 2001; Scagel and Andersen, 1997). The decreased soil respiration is thought to be due to reduced root growth under O_3 exposure, but could also be partially explained by decreased microbial respiration in response to O_3. Additional research is necessary to identify

the role of O_3 in affecting root versus heterotrophic respiration, particularly over long time intervals.

Carbohydrate availability and use influence the degree to which plants respond to O_3 exposure. A model simulation of the effect of O_3 exposure on bole growth of Ponderosa pine showed a 15% reduction in mass (Weber and Grulke, 1995), largely influenced by differences in carbohydrates allocated and partitioned in repair processes elsewhere in the tree. Foliar respiration is thought to increase under elevated O_3 because maintenance costs (energy needs) of leaves damaged by O_3 are higher than normal (Grulke and Balduman, 1999; Noormets et al., 2001b). However, differences in foliar respiration are subtle and difficult to detect statistically. Foliar carbohydrate studies also suggest that more C is used under O_3 stress for repair processes (Grulke et al., 2001; Topa et al., 2001) which would result in increased respiration. Ozone exposure also reduced enzymatic activities of carbohydrate metabolism related to the breakdown of sucrose (Einig et al., 1997). Changes in soil respiration in response to O_3, even though O_3 does not penetrate into the soil, illustrates the tight coupling of plant C balance and soil biota and illustrates the potential role O_3 plays in altering ecosystem C balances (Andersen, 2000).

Ozone can affect plant allometry through changes in energy use, potentially affecting net primary production (NPP) at larger scales. The net effect of O_3 impacts on photosynthesis and respiration for sensitive components of natural ecosystems is that height growth (Isebrands et al., 2001; Oksanen, 2003a) and radial growth (Isebrands et al., 2001; Oksanen, 2003b; Peterson et al., 1987, 1991) can be negatively affected by O_3. This has been extrapolated to decreased NPP (Hogsett et al., 1997; Laurence et al., 2000).

Energy flow in plant communities can be altered by O_3 through changes in C allocation. It is well known that elevated O_3 affects C allocation to roots (Andersen et al., 1997; Coleman et al., 1995b; Grulke et al., 1998a, 2001; Grulke and Balduman, 1999) by decreasing or inhibiting phloem loading of carbohydrates (Grantz and Farrar, 1999; Landolt et al., 1997), or of carbohydrate metabolism (Einig et al., 1997). This leads to depressed root growth (Andersen et al., 1991; Coleman et al., 1996; Grulke et al., 1998a) and the potential for plant communities to have an increased susceptibility to drought through altered root-shoot balance. Furthermore, it can negatively affect below-ground food webs (Phillips et al., 2002; Scagel and Andersen, 1997).

Another energetically costly response to O_3 exposure is that the production of defense compounds, such as antioxidants, tend to increase under elevated O_3 conditions (Sheng et al., 1997; Tausz et al., 1999c, 2002). Antioxidants help the plant scavenge free radicals before they can cause damage to membranes or cell walls, but they demand C for production such that growth can be adversely affected. In mature Jeffrey pine, stomatal uptake of O_3 elicited one complex of antioxidant defenses in mesic microsites, while endogenously generated free radicals in the chloroplast elicited a second complex of antioxidant defenses in xeric microsites (Grulke et al., 2003b).

AX9.6.7.2 Material Flow

Plants as producers are responsible for using inorganic atmospheric C and reducing it into organic forms used by consumers, thus driving nutrient processes in ecosystems. Ozone has the potential to disrupt material flow through organic C cycling and changes in nutrient cycling, particularly N and P cycling. Although there is indirect evidence that O_3 is disrupting C and nutrient cycling at the ecosystem level, there is little direct evidence that O_3 alters nutrient processing at ecosystem scales.

The greatest annual nutrient and C input to ecosystems is from foliar and root turnover. Excision of plant parts and whole plant mortality are potentially much larger, but syncopated, ecosystem inputs. Ozone exposure alters C cycling in the ecosystem by affecting the within-plant C allocation and partitioning in dominant, O_3-sensitive plants, and through chemical composition and rate of decomposition of sloughed plant parts (roots, branches, leaves) (Figure AX9-19).

In addition to O_3-induced changes in the quantity of C and nutrient inputs into ecosystems, O_3 also can alter the nutrient quality of inputs. Ozone exposure alters nutrient levels in the foliage (Boerner and Rebbeck, 1995; Fenn and Poth, 1998; Lindroth et al., 2001; Momen et al., 2002) and affects the C:N ratio (Andersen et al., 2001; Grulke et al., 2003b; Grulke and Lee, 1997; Lindroth et al., 2001). Concentrations of compounds such as tannins, lignin, and phenolics (Baumgarten et al., 2000; Findlay et al., 1996; Kim et al., 1998; Saleem et al., 2001) are also affected by O_3 exposure, which in turn alters decomposability (Fenn and Dunn, 1989) and litter buildup in the ecosystem.

There are several possible pathways by which O_3 may affect litter quality and, hence, litter decomposition, thus altering nutrient flow in ecosystems. These include altered C quality, altered nutrient quality, and alteration of leaf surface organisms important in decomposition pathways. For example, yellow poplar and black cherry litter exposed to O_3 showed greater N loss during decomposition than charcoal-filtered controls, although mass loss did not vary among O_3 treatments (Boerner and Rebbeck, 1995). Subsequent studies showed that although foliar N was not affected by O_3 exposure in yellow poplar leaves, foliage decomposed more slowly (Scherzer et al., 1998). Other studies have also shown a change in foliar N concentration in response to O_3 treatment, affecting the C:N ratio and possible litter quality (Andersen et al., 2001).

In some cases, it appears that N remobilization from foliage into the plant is not complete at the time of foliage abscission in O_3-exposed plants (Findlay and Jones, 1990; Matyssek et al., 1993; Patterson and Rundel, 1995; Stow et al., 1992). Greater N content of senesced litter could increase rates of decomposition. When O_3-exposed cottonwood (*Populus deltoides*) leaves abscissed at the same time as control leaves, they decomposed at similar rates; however, prematurely senesced foliage from O_3-exposed cottonwood decomposed more slowly than controls despite their higher N content (Findlay et al., 1991; Findlay and Jones, 1990). Higher N in senesced leaves appeared to be related to organic complexes formed by bound phenolics in O_3-exposed leaves, making the litter less palatable to decomposers, and slowing decomposition rates (Findlay et al., 1996; Jones et al., 1994). Increased phenolics also have been found in European silver birch (*Betula pendula*) exposed to O_3 (Saleem et al., 2001).

Carbon quality in leaf litter also changes in O_3-exposed foliage. Compositional changes in leaf structural characteristics, such as lignin content, would be expected to alter rates of litter decomposition (Fogel and Cromack, 1977; Kim et al., 1998; Meentemeyer, 1978). Blackberry litter exposed to elevated O_3 had greater permanganate lignin than the control treatment, a difference that was inversely related to mass-loss rates in decomposition studies (Kim et al., 1998).

Ozone may affect early stages of decomposition by altering populations of leaf surface organisms before or after senescence. Magan et al. (1995) found a shift in phyllosphere fungi on Scots pine, Sitka spruce (*Picea sitchensis*), and Norway spruce exposed to O_3, but the potential effect of these changes on subsequent litter decomposition was uncertain. The slowest

decomposition rates of preexposed blackberry leaves were found when senesced foliage was exposed to O_3 during decomposition, suggesting a possible direct effect of O_3 on microorganisms in decomposing litter (Kim et al., 1998). Whether O_3 concentrations at the soil surface influence initial stages of litter decomposition remains to be addressed.

Ozone exposure also reduces nutritional content of foliage because of the degradation of chlorophyll. Resynthesis of chlorophyll may be limited by nutritionally poor soils or low soil moisture, as well as alteration of root uptake by O_3 exposure and other stressors. Foliar exposure to O_3 may also increase leaching of nutrients (Kerstiens and Lendzian, 1989). Ozone exposure promotes early senescence of foliage (Heath and Taylor, 1997; Miller and Elderman, 1977), with higher nutrient content than if excised later in the growing season (Poth and Fenn, 1998).

Since O_3 can slow decomposition through changes in leaf quality and quantity, leaf litter can accumulate (Fenn and Dunn, 1989). The accumulation of soil organic matter from increased leaf litter, even in the absence of acidic deposition, can lower soil pH (Binkley, 1992). Lower soil pH can promote loss of nutrients, particularly cations, from the system, further reducing nutrient availability to the plant. Complex organic compounds in decomposing litter may also tie up nutrients, rendering them less available to plants.

Other factors such as nutrient deposition also affect the degree to which O_3 influences C and nutrient flow through ecosystems. In high-pollution sites, the effect of N deposition is difficult to separate from O_3 exposure, and reviews of the effect of acidic (and N) deposition on ecosystem nutrient dynamics are important to consider (see Binkley [1992]; Fenn et al. [1998, 2003b]). For example, at moderately high pollution sites, foliar content of N is higher than that at lower pollution sites, but so are P, Mg, and Fe contents (Poth and Fenn, 1998). Although significant changes in foliar tissue chemistry have occurred in response to long-term pollutant deposition in the Carpathians (Fenn et al., 2002; Mankovska et al., 2004), much of this response is correlated to heavy metal and N and S deposition. At this point, the contribution of O_3 exposure alone cannot be isolated without careful between-site comparisons of plant response and understanding the depositional velocities of constituent atmospheric species. The significant effects of plant response along known pollution gradients are important to consider, because heavy metal and N and S deposition has significantly declined over the last decade, while O_3 exposures remain high and declines in forest health have been sustained.

Models provide a means to track material flows through ecosystems. Two biogeochemical models were parameterized to capture long-term effects of O_3 exposure, N deposition, and climate on a Ponderosa pine-dominated site in the eastern San Bernardino Mountains. Simulated O_3 exposure resulted in faster production and turnover of foliage and a shift in C from the canopy (15% reduction) to the forest floor (increase of 50 to 60%) (Arbaugh et al., 1999). When O_3 exposure was combined with that of N deposition, litter mass increased exponentially.

The direct effect of O_3 exposure on below-ground nutrient dynamics and ecosystem material flow is poorly understood. Additional research will be necessary to understand spatial and temporal dynamics of nutrient and C flow in ecosystems and to separate the effects of O_3 from those attributable to N deposition.

AX9.6.8 Hydrological and Geomorphological

At present, there are no publications on the effects of O_3 exposure that are carried through at the ecosystem level to changes in mass water flow, channel morphology, riparian habitat complexity, or sediment movement. It is possible that processes occurring at smaller scales are affecting geomorphological processes in ecosystems; however, difficulty in scaling these responses spatially and temporally have made it difficult to show experimentally. It is possible that O_3 exposure affects water quality through changes in energy and material flows, as discussed previously.

AX9.6.9 Natural Disturbance Regimes

There has been little research on how natural disturbances interact with O_3 to affect performance of plants, communities, and ecosystems. The frequency, intensity, extent, and duration of natural disturbances are variable and unpredictable. However, there have been enough ecophysiological studies to suggest that O_3 could predispose plant communities to certain natural stresses, e.g., drought stress or extreme low-temperature stress during the winter.

While several studies have shown that drought stress reduces O_3 uptake through stomatal closure, evidence also suggests that O_3 can alter plant water use and susceptibility to drought. In controlled studies, Reich and Lassoie (1984) showed that relatively low O_3 concentrations could diminish stomatal control and alter water use efficiency. Ash trees (*Fraxinus excelsior*) exposed to elevated O_3 had greater water use early in the growing season, but less water use late in the

growing season when exposed to elevated O_3 (Wiltshire et al., 1994). Under moderate drought stress, Norway spruce trees grown under elevated O_3 consumed water faster and showed higher stomatal conductances than controls (Karlsson et al., 1995). Pearson and Mansfield (1993) showed that successive O_3 episodes disrupted stomatal function, making beech seedlings more susceptible to drought. Previous year O_3 exposure was shown to have a carry-over effect in the following growing season for beech (Pearson and Mansfield, 1994).

Few studies showing the effects of O_3 on water relations of field-grown trees are found in the literature. However, Grulke et al. (2003a) examined the effects of O_3 on canopy transpiration of Jeffrey pine from mesic and xeric microsites and found that trees from mesic sites had 20% more O_3 uptake than those in the xeric sites. The authors also concluded that the mesic trees had greater O_3 injury as evidenced by lower needle retention, whereas trees in xeric microsites had greater chlorotic mottle. Chlorotic mottle induced by stomatal uptake of O_3 is indistinguishable from that of endogenously produced oxidants resulting from partially closed stomata, a reduction of CO_2 inside the leaf, and production of strong oxidizers within the chloroplast when excited electrons are passed to O_2 instead of CO_2 under high light levels.

Trees living near the limits of their freezing-tolerance range may be especially susceptible to predisposition of freezing injury by O_3 (Sheppard et al., 1989). However, Aleppo pine exposed to elevated O_3 had enhanced winter hardiness (Wellburn and Wellburn, 1994). As with the seasonal carryover of drought susceptibility, the influence of elevated O_3 on freezing tolerance is carried over from summer to winter. Such effects have been demonstrated for Sitka spruce (Lucas et al., 1988) and for red spruce (Waite et al., 1994). Sorting out the role of elevated O_3 in contributing to frost or low-temperature damage in forests remains difficult due to the presence of other factors that may affect senescence.

AX9.6.10 Scaling to Ecosystem Levels

The vast majority of literature describing O_3 effects comes from short-duration herbaceous plant or tree seedling studies under controlled conditions. Scaling results from these studies requires extrapolation over both space and time in order to understand the full extent of changes in ecosystems. In addition to spatial, temporal, and age-related complexities, ecosystems are composed of organisms whose lifetimes range from hours to centuries (Laurence and Andersen, 2003). Forested ecosystems are affected by environmental conditions such as water and nutrient

availability, as well as by intra- and interspecies competition. Therefore, direct experimentation to determine the response of forested ecosystems is not simply a matter of determining the effect of O_3 on individual mature trees. In addition, even if an experiment can be conducted, extrapolation of the results across landscapes and regions remains challenging. Nonetheless, models provide a means to explore possible long-term changes and to identify important research uncertainties.

Approaches to scaling fall roughly into two categories: (1) process-based modeling to extrapolate physiological responses to O_3 based on seedling studies and (2) field assessments using surveys and growth correlations, often in association with stand-level models to address ecosystem complexity. Comparatively good information is available on process level effects of O_3 in seedlings and, therefore, some models offer the opportunity to use this information to scale O_3 effects at the stand and regional scales (Chappelka and Samuelson, 1998; Fuhrer et al., 1997; Hogsett et al., 1997; Laurence et al., 2000). Field assessments offer the opportunity to examine larger plot sizes, older trees, and trees growing under realistic competition. These two approaches are discussed in more detail below.

AX9.6.10.1 Scaling from Seedlings to Mature Trees

A number of investigators have used simulation models based on physiological processes to integrate available data and predict the effects of O_3 on mature trees. Such models predict tree growth by simulating fundamental mechanisms, rather than through a statistical analysis of empirical data. For instance, the process of photosynthesis is simulated based on environmental conditions and physiological characteristics, and then the fixed C is allocated to plant growth using principles of plant physiology. Models based on mechanisms should be applicable across wide areas if the important functional relationships are represented accurately in the models and if the environmental conditions are accurately identified. The ability of six models (TREGRO, CARBON, ECOPHYS, PGSM, TREE-BGC, and W91) to simulate the effects of climatic change and O_3 have been reviewed by Constable and Friend (2000). Of these models, only PGSM and TREGRO explicitly simulated the effects of O_3 on foliar processes.

The TREGRO model was used to simulate C allocation and tissue growth in seedlings and mature red oak trees based on the experimental data discussed above (Weinstein et al., 1998). For seedlings at 2×-ambient O_3, only the total nonstructural carbohydrate (TNC) storage pool

was predicted to be affected. For mature trees, large decreases were predicted for TNC, leaves, stem, branch, and both fine and coarse roots. Most predicted effects in mature trees were consistent with observations in the field, but the simulations overestimated the effect of 2×-ambient O_3 on root TNC and growth. The authors suggested that this discrepancy may have been due to trees reducing respiration in response to O_3 stress, a response not simulated in the model.

For *Abies concolor*, TREGRO was parameterized and simulated growth of a mature tree for 3 years to test for effects of O_3 exposure and drought stress (Retzlaff et al., 2000). Reductions in O_3 exposure-mediated carbon assimilation were translated to losses in whole tree biomass that probably would not be detectable in the field. However, TNC levels in branch tissue were simulated to be lowered by over 50%, and branch growth was reduced in a moderately polluted site relative to a clean site. Low O_3 exposure (sufficient to decrease C assimilation by 2.5%) and drought stress (25% reduction in annual precipitation, which is common on a decadal scale) acted synergistically to reduce C gain of whole tree biomass of *A. concolor*. Simulated results of the tests were comparable to effects found in OTCs for seedlings and pole-sized trees in clean and moderately polluted sites.

Models such as TREGRO are usually parameterized from many different sources of data, including chamber experiments and plantations, from seedlings to mature trees, making it difficult to validate that they reproduce changes that occur as trees develop from seedlings to maturity. To address this issue, physiological and growth data were collected from a natural stand of *P. ponderosa* and used to parameterize the TREGRO model (Grulke and Retzlaff, 2001). Representative trees of each of five tree age classes were selected based on population means of morphological, physiological, and nearest neighbor attributes. Seedlings were observed to differ significantly from pole-sized and older trees in most physiological traits. The changes in biomass with tree age predicted from the model closely matched those of trees in the natural stand.

The PGSM model was used to simulate *Pinus ponderosa* seedling growth responses to O_3 exposure and drought stress (Chen et al., 1994). Drought stress was predicted to reduce the effect of O_3 on growth, as was observed in the experimental data. The TREGRO model was used to simulate responses of Pacific Coast and interior varieties of *P. ponderosa* to five simulated O_3 exposures between subambient and 3×-ambient (Constable and Taylor, 1997).

Simulated growth of var. *ponderosa* was reduced more than that in var. *scopulorum* with all O_3 exposures. Drought was protective of O_3 exposure. Similar results were also found in a relatively moist Ponderosa pine plantation (Panek and Goldstein, 2001), whereas drought was synergistically deleterious with cumulative O_3 exposure in a natural stand (Grulke et al., 2002b).

For *Pinus taeda*, the Plant Growth Stress Model (PGSM) was calibrated with seedling data and then used to simulate the growth of mature trees over a 55 year period in the Duke Forest, NC, using estimates of historical O_3 concentrations (Chen et al., 1998). Simulated stem diameter and tree height were comparable to observed values. In another simulation using TREGRO, loblolly pine was more sensitive (greatest reduction in C gain) to a peak O_3 episode in July (Constable and Retzlaff, 1997), whereas mature yellow poplar *(Liriodendron tulipifera*) was more sensitive to a peak O_3 episode in August.

For *Populus tremuloides*, the ECOPHYS model was used to simulate the relative above-ground growth response of an O_3-sensitive clone (259) exposed to square-wave variation in O_3 concentration (Martin et al., 2001). The model adequately simulated several effects of O_3, including a greater effect on stem diameter than on stem height, earlier leaf abscission, and reduced stem and leaf dry matter production at the end of the growing season. For *Acer saccharum*, the TREGRO model was use to predict effects of a 10-year O_3 exposure on root and stem growth of a simulated 160-year-old tree (Retzlaff et al., 1996). Twice-ambient O_3 exposure (for Ithaca, NY) was predicted to deplete the TNC pools and reduce fine root production.

AX9.6.10.2 Surveys, Growth Correlations, and Stand-Level Modeling

Stand-level studies have included surveys of O_3 symptoms, correlations of radial growth with O_3 and other environmental factors, and regional-scale modeling. In addition, open air O_3 exposure systems, such as those being used on *Populus tremuloides*-mixed stands in northern Wisconsin (Karnosky et al., 2003b) and on *Fagus sylvatica* and *Picea abies* in Germany (Nunn et al., 2002) offer an opportunity to examine larger plot sizes, older trees, and trees growing under realistic competition. Plots along natural O_3 gradients, as have been used very effectively in southern California forest studies (Miller and McBride, 1999b) and with *P. tremuloides* stands in the Great Lakes region (Karnosky et al., 1999), offer additional insights into ecosystem level responses. Undoubtedly, however, simulation modeling will have to become an integral component of research in order to predict adequately ecosystem responses to O_3 (Laurence and

Andersen, 2003). Results of these approaches are discussed below, organized into three U.S. regions: (1) northern states (including the upper Midwest and the Northeast), (2) southeastern states, and (3) western states (primarily California). A fourth section contains selected information from Europe.

Northern and Midwestern United States. In recent years, the USDA Forest Service has conducted systematic O_3 biomonitoring surveys in most north-central and northeastern states (Coulston et al., 2003; Smith et al., 2003). Plots are located on a systematic grid, and trained field crews evaluate up to 30 plants of up to six species that have foliar injury symptoms diagnostic of O_3 damage. For the United States as a whole, injury has been found more often in eastern than in interior or west-coast states. As expected, O_3 injury is more common and more severe in areas with higher O_3 concentrations. Of sampled *Prunus serotina* plots, ~12% were estimated to be at high risk for injury based on a injury index derived from the survey data (Coulston et al., 2003). *P. serotina* was estimated to be at risk for injury on the Allegheny Plateau and the Allegheny Mountains (in Pennsylvania, West Virginia, and Maryland), as well as in the coastal plain of Maryland and Virginia.

Ozone concentration, foliar injury, and physiological traits were measured on *P. serotina* trees of different sizes in Pennsylvania (Fredericksen et al., 1995). The proportion of foliage injured was 46% for seedlings, 15% for saplings, and 20% for canopy trees. Cumulative O_3 flux was the most useful O_3 metric for predicting injury. Injury was negatively correlated ($r^2 = 0.82$) with net photosynthetic rates, but was not related to stomatal conductance. *P. serotina* is discussed further below, because detailed surveys have been conducted in the Shenandoah and Great Smoky Mountains NPs in the southeastern United States.

Over the past several decades, some surveys of white pine (*Pinus strobus*) have reported significant associations between foliar injury and reduced growth (Anderson et al., 1988; Benoit et al., 1982). However, a review of 93 surveys conducted from 1900 through the late 1980s concluded that methodological problems were pervasive, including such issues as proximity to roads, lack of peer review, lack of random sampling, small sample sizes, and lack of quantitative methods to estimate severity (Bennett et al., 1994). Because of these problems, along with evidence of adequate growth rates for *P. strobus* regionally and contradictory evidence from numerous studies of symptom production in response to controlled O_3 exposure, these authors

concluded that there was no clear evidence of decline in *P. strobus* (Bennett et al., 1994). A more recent study in Acadia National Park (ANP) in Maine found no association between O_3 exposure in OTCs and symptom development in *P. strobus*, calling into question whether symptoms previously ascribed to O_3 may be caused by some other stress (Kohut et al., 2000). However, another ANP study found significant correlations between O_3 exposure and the radial growth of trees during 10 years in 7 of 8 stands examined (102 trees total; (Bartholomay et al., 1997). Taken together, these results suggest that there may not be an association between growth of *P. strobus* trees and putative O_3 symptoms, but there may be an association between O_3 exposure and radial growth of mature trees in the field.

The study of O_3 effects was undertaken from 1990 to 1993 in the ANP in Maine, because this location experiences elevated O_3 exposures due to transport from urban areas located upwind (Kohut et al., 2000). Thirty-two species of plants found in the park were propagated and exposed to O_3 in OTCs. In addition, ambient O_3 concentrations were monitored at the study site at 15 m above sea level and near the top of Cadillac Mountain at 470 m above sea level. At the study site, the maximum 1-h O_3 concentration was 140 ppb, which occurred in both 1990 and 1991. Daytime 12-h O_3 concentrations were 35, 41, 36, and 37 ppb during the four years; and O_3 concentrations were consistently higher at the high-elevation site. Species showing foliar injury at ambient O_3 concentrations included black cherry (*Prunus serotina*), quaking aspen (*Populus tremuloides*), white ash (*Fraxinus americana*), jack pine (*Pinus banksiana*), big-leaf aster *(Aster macrophyllus*), and spreading dogbane (*Apocynum androsaemifolum*). Species showing foliar injury at 1.5 × ambient O_3 concentrations included grey birch (*Betula populifolia*), small sundrops (*Oenothera perennis*), and bunchberry *(Cornus canadensis*). Species remaining uninjured at 2 × ambient O_3 concentrations included paper birch (*Betula papyrifera*), white pine (*Pinus strobus*), pitch pine (*Pinus rigida*), red spruce (*Picea rubens*), Eastern white cedar (*Thuja occidentalis*), English oak (*Quercus robur*), Canada bluejoint grass (*Calamagrostis canadensis*), wild radish (*Raphanus raphanistrum*), and Canada mayflower (*Maianthemum canadense*). Because of their O_3 sensitivity and diagnostic symptoms, big-leaf aster, spreading dogbane, quaking aspen, white ash, and black cherry were recommended as bioindicators for the ANP.

The PnET-II model was applied to 64 locations across the northeastern United States to simulate the effects of ambient O_3 on mature hardwood forests (Ollinger et al., 1997). In this model, O_3 effects on each of several layers of the forest canopy were represented by a single

linear equation relating predicted O_3 uptake to decreased net photosynthetic rate. Wood growth was predicted to decrease between 3 to 22%, with greatest reductions in southern portions of the region where O_3 levels were highest and on soils with high water-holding capacity where drought stress was absent. Little variation was predicted among years, because high O_3 often coincided with hot, dry weather conditions that reduced predicted stomatal conductance and O_3 uptake.

In order to estimate the impact of O_3 on forests, effects must be evaluated not only on individuals, but also on mixtures of species and the composition of forest stands. The PnET model described above evaluated the effects of O_3 on broad forest types (an evergreen/deciduous mix), but did not address specific forest species composition. In order to address competition among species, the TREGRO model was linked to the ZELIG forest stand growth model and a geographic information system was created to predict the effects of O_3 across the north-central and northeastern United States (Laurence et al., 2000). ZELIG is a gap-succession model used to simulate succession in mixed stands typical of eastern and northern forests. Ambient O_3 generally caused a reduction of 2 to 4% in the growth of *Quercus rubra* across the region during the 100-year simulation. The response followed the pattern of O_3 exposure, with little effect in the northwest part of the region, but with greater effect in southern locations. The O_3 response of *Acer saccharum* to O_3 varied widely, but the overall growth response was always positive, indicating that the evergreen/deciduous mix was able to take advantage of the decrease in the growth of *Q. robur* and other species caused by O_3. In the northernmost part of the region, *A. saccharum* growth increased by up to 3%, but in the southern part of the region, its growth increased by up to 12%. The authors ascribed this enhanced growth to a combination of warmer temperatures and reduction in the growth of *Prunus serotina*, a minor component of the simulated stand that was very sensitive to O_3.

Southeast United States. In a survey of the Great Smoky Mountains NP, foliar injury attributed to O_3 was found on 47% of the more than 1,600 plants examined (Chappelka et al., 1997). In subsequent surveys of injury in the park, injury was found on mature trees of the following species: *Sassafras albidum*, *Prunus serotina*, and *Liriodendron tulipfera* (Chappelka et al., 1999a,b). In a similar study in Shenandoah National Park, injury was found on *Fraxinus americana*, *P. serotina*, and *L. tulipfera* (Hildebrand et al., 1996).

For *Prunus serotina* seedlings grown in soil in OTCs and exposed to relatively low ambient levels of O_3 in Pennsylvania, there was no correspondence between visible foliar stipple, leaf gas exchange, and seedling growth between two families previously shown to differ in O_3 symptoms (Kouterick et al., 2000). However, significant exposure-response relationships were found for foliar injury in the Great Smoky Mountains and Shenandoah National Parks. In each park, foliar injury was evaluated on mature *P. serotina* trees on three plots at different elevations near O_3 monitors during 1991 to 1993 (Chappelka et al., 1999a,b; Hildebrand et al., 1996). In 1991, incidence was 60% and 45% for the two parks and 33% in both parks during 1992 and 1993. Symptoms were greater at the highest elevations where O_3 concentrations were highest. In another study, radial growth rates were measured in 44 *P. serotina* trees ranging in age from 19 to 56 years old with and without O_3 symptoms, at three sites in the Great Smoky Mountains National Park. Trees with O_3 symptoms were compared to similar-sized trees with few symptoms. There was no evidence that trees with O_3 symptoms had lower growth rates ($p = 0.6$) (Somers et al., 1998).

In the Great Smoky Mountains National Park, radial growth rates were measured for 44 *L. tulipfera* trees ranging in age from 30 to 58 years old, with and without O_3 symptoms, at three sites at different elevations (Somers et al., 1998). Trees with O_3 symptoms averaged 30% lower growth rates over ten years ($p = 0.0005$). Seedlings of *Liriodendron tulipfera* were exposed for two seasons to 2×-ambient O_3 exposures in OTCs in Delaware, OH (seasonal SUM00 exposures of 107 and 197 ppmh) (Rebbeck, 1996). Foliar O_3 symptoms were observed, but growth was not reduced.

In order to evaluate the influence of interspecies competition on O_3 effects, the linked TREGRO and ZELIG modeling system was used to predict the effects of O_3 over 100 years on the basal area of species in a *Liriodendron tulipfera*-dominated forest in the Great Smoky Mountains NP (Weinstein et al., 2001). Ambient O_3 was predicted to reduce the basal area of *L. tulipfera* by 10%, whereas a 1.5×-ambient exposure was predicted to cause a 30% reduction. Basal area of *Acer rubrum* and *Prunus serotina* was predicted to increase for some years, but then decrease by up to 30%, with few changes in the total basal area of all species by the end of the simulation.

In order to evaluate the influence of interspecies competition on O_3 effects, the linked TREGRO and ZELIG modeling system was used to predict the effects of O_3 on the basal area of

Pinus taeda and *Liriodendron tulipfera* growth throughout their ranges (Laurence et al., 2003). The models were parameterized using biological and meteorological data from three sites in the southeastern United States (in Alabama, Louisiana, and North Carolina). Forest stand response to five O_3-exposure regimes with annual SUM06 values ranging from 0 to 100 ppmh per year was simulated for 100 years. The simulated basal area of the two species was generated within the context of four other tree species common in southeastern forests. Basal area of *P. taeda* was highly responsive to precipitation and O_3 exposure, with the greatest increases under high-precipitation, low O_3-exposure scenarios and the greatest decreases under low-precipitation, high O_3-exposure scenarios. The basal area of *L. tulipifera* did not significantly differ (+10%) from simulations using a "base case" (ambient O_3, average precipitation).

Systematic biomonitoring surveys found that approximately 24% of sampled sweetgum and 15% of sampled *Pinus taeda* plots were estimated to be at high risk for foliar injury on the coastal plain of Maryland and Virginia (Coulston et al., 2003; Smith et al., 2003). In a study in Tennessee, the effect of ambient (uncontrolled) O_3 on 28 mature canopy-dominant 50 to 90-year-old *P. taeda* trees in five stands was measured over a 5-year period (McLaughlin and Downing, 1995, 1996). Of many O_3 metrics, a 3-day average of hourly O_3 values $\geq$ 40 ppb (AOT40) was found to best explain short-term variation in stem expansion as measured with dendrometer bands. Interactions between O_3, temperature, and drought stress (as indicated by the weekly moisture stress index) accounted for 63% of the short-term variation in stem growth rates. Because there are interactions among O_3, drought stress, and temperature that may differ with the averaging time (days to years), this type of study cannot provide conclusive proof of cause and effect (Reams et al., 1995). However, the results do suggest that the effects of O_3 measured on loblolly seedlings may also be occurring in mature trees in both wet and dry sites. The magnitude of effects of O_3 on growth, including interactions with other variables in this study, ranged from 0 to 15% over 5 years, with an average of 5.5%.

Western United States. The USDA Forest Service conducted O_3 biomonitoring surveys in Washington, Oregon, and California during one year (1998), and in the Sierra Nevada and Sequoia NFs every other year for several decades (Campbell et al., 2000). Overall, only one plot showed any symptoms of O_3 injury outside of the Sierra Nevada and Sequoia National Forests. In the Sierra Nevada National Forest, between 30 and 40% of trees showed injury from 1989

through 1997. In the Sequoia National Forest, between 40 and 50% of the trees surveyed showed injury from 1990 through 1998.

For *Pinus ponderosa* along a well-studied gradient of O_3 exposure in the San Bernardino mountains, chlorotic mottle was highest on foliage at the most polluted site, as has been found previously (Grulke and Balduman, 1999). Based on whole-tree harvests, root biomass was lowest at the most polluted sites, confirming previous studies with seedlings under controlled conditions, as discussed above. Ozone responses in highly polluted environments such as Southern California may not be predicted adequately by extrapolating effects from single-factor experiments. Instead, combined approaches utilizing field experiments and modeling efforts may be required to properly account for a combination of stressors including O_3, N deposition, and drought. Furthermore, the available studies underscore the lack of correlation between O_3 symptoms and mature tree effects. If a field survey fails to find a correlation between mature tree growth and O_3, this result may be due to the dominant effect of another factor such as N deposition and may not be evidence that O_3 does not reduce the growth of mature trees.

The TREGRO model was used to evaluate how projected future temperature and CO_2 concentrations might affect the response of individual Ponderosa pine to O_3 at seven sites in California, Oregon, and Washington (Tingey et al., 2001). As expected, growth decreased with increasing O_3 exposure. Differences in O_3 response among sites appeared to be due primarily to differences in precipitation.

Often air quality standards do not translate directly into measurable improvements in tree growth or productivity. To evaluate whether past improvements in air quality have improved Ponderosa pine growth, TREGRO was used to simulate growth at sites in the San Bernardino Mountains in California (Tingey et al., 2004). Ozone and meteorological data from the past 37 years was used to run the simulations. Despite variation in precipitation and temperature, O_3 was found to reduce simulated tree growth. The authors were able to simulate growth improvements as air quality improved during the 1980s and 1990s, suggesting that improvements in emission control strategies benefitted Ponderosa pine. The model simulations were qualitatively consistent with improvements in canopy condition that were observed at sites where O_3 reductions were the greatest.

Studies in Europe. In a 4-year study of *Fagus sylvatica* in Switzerland at 57 forest sites ranging in age from 65 to 173 years, stem increment was found to decrease with increasing maximum O_3 exposure (Braun et al., 1999). In this study, O_3 concentration was estimated by interpolation among monitoring stations, and other site conditions such as soil water status and temperature were interpolated from weather stations. Other factors such as N deposition, tree diameter, and canopy dominance were also found to be significantly associated with stem increment. The maximum annual O_3 dose (expressed as AOT40) was found to be more strongly associated with decreased stem increment than was the average O_3 dose over the 4 years. A growth reduction of 22.5% (confidence interval 14.3 to 28.6%) was associated with each 10 ppmh increment of O_3 (expressed as AOT40). This decrease was steeper than the 6.1% growth reduction summarized previously from several OTC studies with *F. sylvatica* seedlings (Fuhrer et al., 1997). However, the authors suggest that this difference may be explained largely by the 4 years of exposure in their forest survey study as compared to the 1-year exposures for seedlings. As with any forest survey, these results must be interpreted with caution because O_3 exposure was correlated with other variables, such as tree age and the deposition of NO_2 and SO_2.

AX9.6.11 Summary of Ecological Effects of Ozone Exposure on Natural Ecosystems

In this chapter, an effort has been made to discuss the adverse effects of O_3 on natural ecosystems within the context of the SAB framework for assessing and reporting ecological conditions (Young and Sanzone, 2002) (Figure AX9-21). Using this framework, there is evidence that tropospheric O_3 is an important stressor of natural ecosystems, with well-documented impacts on the biotic condition, ecological processes, and chemical/physical nature of natural ecosystems. In turn, the effects of O_3 on individual plants and processes are scaled up through the ecosystem affecting processes such as energy and material flow, intra- and interspecies competition, and NPP. Thus, effects on individual keystone species and their associated microflora and fauna may cascade through the ecosystem to the landscape level. This suggests that by affecting water balance, cold hardiness, tolerance to wind, and by predisposing plants to insect and disease pests, O_3 may even influence the occurrence and impact of natural disturbance. Despite the probable occurrence of such effects, however, there are essentially no

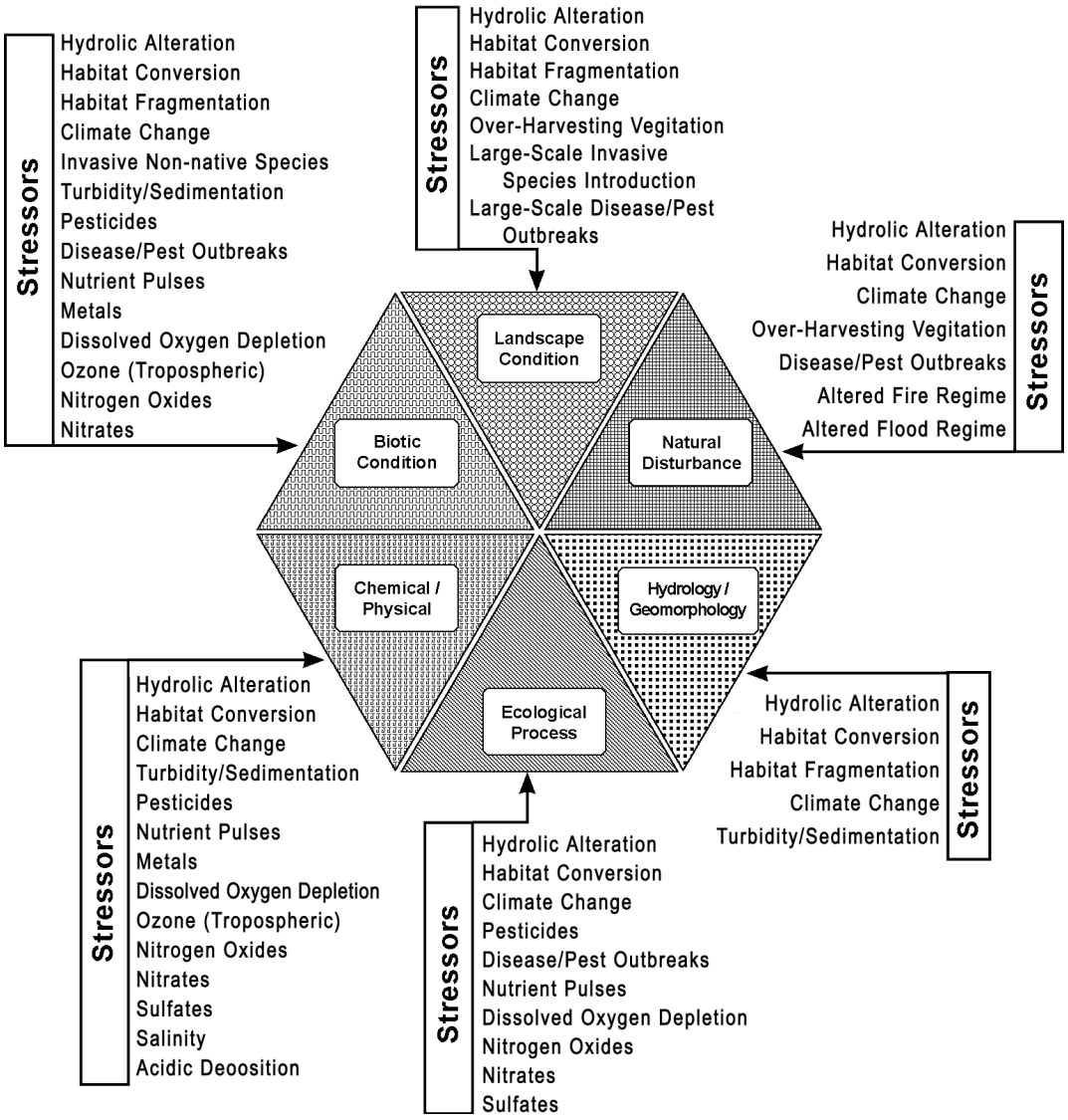


Figure AX9-21. Common anthropogenic stressors and the essential ecological attributes they affect.

Source: Modified from Young and Sanzone (2002).

instances where highly integrated ecosystem-level studies have conclusively shown that O_3 is indeed altering ecosystem structure and/or function.

Systematic injury surveys demonstrate that foliar injury occurs on O_3-sensitive species in many regions of the United States. However, the frequent lack of correspondence between foliar symptoms and growth effects means that other methods must be used to estimate the regional

effects of O_3 on tree growth rates. Investigations of the radial growth of mature trees, in combination with data from many controlled studies with seedlings, as well as a few studies with mature trees suggest that ambient O_3 is reducing the growth of mature trees in some locations. Studies using models based on tree physiology and forest stand dynamics suggest that modest effects of O_3 on growth may accumulate over time and may interact with other stresses. For mixed-species stands, such models predict that overall stand growth rate is generally not likely to be affected. However, competitive interactions among species may change as a result of growth reductions of sensitive species. These results suggest that O_3 exposure over decades may be altering the species composition of forests in some regions.

RESEARCH NEEDS

The knowledge base for examining the range of ecological effects of O_3 on natural ecosystems is growing, but significant uncertainties remain regarding O_3 effects at the ecosystem level. For example, there is a need for information on the following ecosystem-level responses:

- *Ecosystem Processes*. Little is known about the effects of O_3 on water, C, and nutrient cycling, particularly at the stand and community levels. Effects on belowground ecosystem processes in response to O_3 exposure alone and in combination with other stressors are critical to projections at the watershed and landscape scales. Little is yet known about the effects of O_3 on structural or functional components of soil food webs, or how these impacts could affect plant species diversity (Andersen, 2003).

- *Biodiversity and Genetic Diversity*. The study of genetic aspects of O_3 impacts on natural ecosystems has been largely correlational in nature and it remains to be shown conclusively whether O_3 affects biodiversity or genetic diversity (Davison and Barnes, 1998; Pitelka, 1988; Winner et al., 1991). Studies of competitive interactions under elevated O_3 levels are needed (Laurence and Andersen, 2003), and reexamination via new sampling of population studies to bring in a time component to previous studies showing spatial variability in population responses to O_3 are needed. These studies could be strengthened by modern molecular methods to quantify impacts on diversity.

- *Natural Ecosystem Interactions with the Atmosphere*. Little is known about feedbacks between O_3 and climate change on volatile organic compound (VOC) production, which in turn, could affect O_3 production (Fuentes et al., 2001). At moderate-to-high O_3 exposure sites, aberrations in stomatal behavior could significantly affect individual tree water balance of sensitive trees, and if the sensitive tree species is dominant, hydrologic balance at the watershed and landscape level could be affected. This has not been addressed in any model because O_3 exposure effects, if included in the modeling effort have assumed a linear relationship between assimilation and stomatal conductance.

- *Below-Ground Interactions*. While the negative effects of O_3 on below ground growth are well characterized, interactions of roots with the soil or microorganisms are not.

- *Other Interactive Effects*. Interaction studies with other components of global change (e.g.,warming, increasing atmospheric CO_2, N deposition, etc.) or with various biotic stressors are needed to better predict complex interactions likely in the future (Laurence and Andersen, 2003). Whether O_3 will negate the positive effects of an elevated CO_2 environment on plant carbon and water balances is not yet known; nor is it known if these effects will scale up through the ecosystem. How might O_3 affect the progress of pest epidemics and insect outbreaks as concentrations increase is unclear (Ball et al., 1998).

- *Reproduction Effects*. Information concerning the impact of O_3 on reproductive processes and reproductive development under realistic field or forest conditions are needed as well as examination of reproductive effects under interacting pollutants (Black et al., 2000).

- *Comparative Extrapolation*. The vast majority of O_3 studies of trees have been conducted with young, immature trees and in trees that have not yet formed a closed canopy. Questions remain as to the comparability of O_3 effects on juvenile and mature trees and on trees grown in the open versus those in a closed forest canopy in a competitive environment (Chappelka and Samuelson, 1998; Kolb and Matyssek, 2001; Samuelson and Kelly, 2001).

- *Scaling-Up Issues*. Scaling the effects of O_3 from the responses of single or a few plants to effects on communities and ecosystems is a complicated matter that will require a combination of manipulative experiments with model ecosystems, community and ecosystem studies along natural O_3 gradients, and extensive modeling efforts to project landscape-level, regional, national and international impacts of O_3. Linking these various studies via impacts on common research quantification across various scales using measures of such factors as leaf area index or spectral reflective data, which could eventually be remotely sensed (Kraft et al., 1996; Panek et al., 2003), would provide powerful new tools for ecologists.

- *Comparative Risk Assessment Methodologies*. Methodologies to determine the important values of services and benefits derived from natural ecosystems such that these could be used in comprehensive risk assessment for O_3 effects on natural ecosystems (Heck et al., 1998).

AX9.7 ECONOMIC EVALUATION OF OZONE EFFECTS ON AGRICULTURE, FORESTRY, AND NATURAL ECOSYSTEMS

AX9.7.1 Introduction

The adverse consequences of ambient air pollutant exposures on vegetation, ecosystems, and components of the material environment have been documented since the beginning of the industrial revolution. Attempts to quantify the monetary damage and injury resulting from tropospheric O_3 exposures to managed agriculture, forests, and natural ecosystems date back at least to the 1950s.

Both methodological and data problems plagued early efforts to assess the monetary damages of air pollution to crops and natural vegetation. Adams and Crocker (1989) discussed the methodological issues, e.g., a lack of reliable data on effects from air pollutants on crop yields or the failure to develop and apply appropriate economic models. Some of these problems were remedied by the EPA's National Crop Loss Assessment Network (NCLAN) in the 1980s. The EPA's NCLAN facilitated the performance of economic assessments by providing O_3-crop yield data with which to estimate O_3-crop yield response functions (see Heagle [1988] for a review of NCLAN procedures and findings). NCLAN also funded a series of economic assessments that, along with subsequent economic assessments, documented substantial economic damages to agriculture. (See Spash [1997] for a detailed review of economic assessments, many of which used NCLAN data.)

Since the completion of the NCLAN program in the late 1980s, the number of economic assessments of air pollution studies focusing on terrestrial ecosystems in general, and agriculture in particular, has declined. For example, for the period of 1980 to 1990, 33 economic studies of O_3 and other air pollutant effects on U.S. crops were published in peer-reviewed journal outlets (Spash, 1997). However, in preparing this section of the current O_3 AQCD, only four peer-reviewed economic assessments were found for the decade of 1991 to 2000 that addressed vegetation in the United States. In addition, one peer-reviewed article (Kuik et al., 2000) was found dealing with agriculture in the Netherlands. Recent interest in global climate change, and the potential effects of global warming on O_3 and other photochemical oxidants, has renewed interest in the effects of air pollution on both managed and unmanaged terrestrial ecosystems (Adams et al., 1998). In addition, concern is growing regarding the effects of air pollutants on natural ecosystems and on the services they provide (Daily, 1997). Unfortunately, this interest

has not yet translated into additional peer-reviewed publications addressing O_3 or other air pollutant effects on ecosystems.

This section of the current O_3 criteria document first discusses the availability of economic information and its usefulness in forming environmental policy. Next, economic assessments of air pollution effects and findings from the 1996 AQCD (U.S. Environmental Protection Agency, 1996) are discussed, followed by a synthesis of the limited literature available since the 1996 AQCD with respect to O_3 effects on agriculture, forestry, and ecosystems. Finally, limitations and continuing uncertainties are reviewed. The most fundamental of these is the lack of measurements of the economic effects of air pollution on natural ecosystems. Other issues include the variability of performance in both managed and natural ecosystems under increased climatic and air pollution variability as well as the challenges related to spatial and temporal scales used in performing economic assessments. To date, this set of effects has been sparsely addressed.

AX9.7.2 The Measurement of Economic Information

Economic science is an exercise in deductive logic in which testable hypotheses about the behavior of economic agents (i.e., farmers, consumers, resource owners) and markets are deduced from a body of theory. That body of theory is based on a series of premises proposed by economists and philosophers dating back over two centuries to Adam Smith. These premises gradually evolved into a theoretical foundation primarily dealing with microeconomics and culminating in structural relationships that define the operation of markets. This foundation was first laid out in a comprehensive and rigorous fashion by Alfred Marshall in 1920. Samuelson (1948) formalized these theoretical relationships, resulting in what is sometimes referred to as modern, or neoclassical, economics.

The insights gained from the theoretical foundation of economics helped shape the nature of applied economic, or policy-relevant research. An example of such applied research is when economists seek to measure the economic consequences of air pollution on agriculture. Such an application is described in Adams and Horst (2003), who provided a graphical representation of the measurement of the effects of air pollution on the well-being of producers and consumers. Economic theory is applied to real-world problems when the methods of economics and the need

for data from other disciplines come into play. When estimating the economic effects of an environmental change, economists need an economic model or method that is theoretically consistent, i.e, defensible, as well as data with which to estimate both environmental science and economic relationships for use in the model. Among accepted economic assessment methods, the actual choice is frequently determined by the nature of the problem to be addressed.
It should be noted that the choice of assessment method can affect the type of economic information that is obtained. Even with a given assessment method, results are sensitive to specific data treatment or assumptions (Adams, 1999). For example, some methods only measure effects on a particular group e.g., farmers. Other methods may measure effects across several groups. Thus, one should not expect the magnitudes of damage or benefits to be identical across economic assessments. One should, however, expect that the direction of the effects will be similar.

Once it is established that an assessment meets basic economic criteria, e.g., including human behavioral responses, the selection of the specific economic assessment method is often a relatively minor issue in terms of estimating benefits of air pollution control (or disadvantages of increases in air pollution). Although results differ across approaches, the differences are largely attributable to specific features of the assessment (e.g., whether the natural science data include a particular effect or relationship, whether effects on consumers are measured, and so forth). The nature and quality of the air quality forecasts used in the assessments can greatly influence the sensitivity of the assessments (Adams and Crocker, 1989). This is particularly noticeable when dealing with forecasts of seasonal air pollution changes (Adams et al., 1988). From the standpoint of providing policy guidance, the differences in economic estimates attributable to the assessment methods are often swamped by uncertainty in the natural and physical science forecasts. This has also been noted in recent economic assessments of climate change (Adams et al., 1998). In many settings, the quality of economic assessments of air pollution is likely to be improved more by refining the physical and natural science data used in the assessments than by intensive efforts to fine-tune the assessment techniques (Adams, 1999).

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 O_3 AQCD (U.S. Environmental Protection Agency, 1996) is unambiguous with respect to the adverse effects of tropospheric O_3 on some types of vegetation. For example, the 1996 AQCD noted that findings from the EPA multiyear NCLAN program in the 1980s provided rigorous corroboration of at least two decades of previous research and a century of anecdotal observations which showed that O_3 at ambient levels caused physical damage to plants in general and to important crop species in particular. Specifically, NCLAN established that ambient O_3 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 O_3 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 O_3 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 O_3 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 O_3 effects on forest productivity in the 1996 AQCD was sparse. The few economic assessments of tree or forest effects reported in the 1996 AQCD were confined to evaluations of assumed or hypothetical changes in output, such as board feet of lumber (e.g., Haynes and Adams, 1992). As noted in the 1996 AQCD, O_3 effects on ecosystems and their services had not been measured in any systematic fashion and no peer-reviewed economic assessments were yet reported.

This section first briefly reviews economic assessments drawn from the review in the 1996 criteria document. This review is the benchmark against which recent articles are then discussed in the subsequent section. As was the case in 1996, the discussion of economic valuation of ecosystem effects is generally limited to conceptual and methodological issues, given the continued lack of empirical analyses in this category.

AX9.7.3.1 Agriculture

In view of the importance of U.S. agriculture for both domestic and world consumption of food and fiber, reductions in U.S. crop yields could adversely affect human welfare. The plausibility of this premise has resulted in numerous attempts to assess, in monetary terms, the losses from ambient O_3 exposures, or the benefits of O_3 control, to agriculture. Twenty-three assessments of the economic effects of O_3 exposures on agriculture were reviewed in the 1996 AQCD, highlighting key issues in the validity of these assessments (U.S. Environmental Protection Agency, 1996). First, the evidence should reflect how crop yields will respond under actual field conditions to O_3 exposures. Second, the air quality data used to frame current or hypothetical effects of O_3 on crops should represent actual exposures sustained by crops at individual sites or production areas. Finally, the assessment methodology into which such data are entered should (1) capture the economic behavior of producers and consumers as they adjust to changes in crop yields and prices that may accompany changes in O_3 air quality; (2) accurately reflect institutional considerations, such as regulatory programs and income support policies (e.g., provisions of federal Farm Bill legislation), that may result in market distortions; and (3) use measures of well-being that are consistent with economic principles.

Assessments of O_3 damage to agricultural crops reported in the 1996 AQCD employed procedures for calculating economic losses that met the conditions described above. More specifically, the assessments provided 23 quantitative estimates of the economic consequences of exposures to O_3 and other air pollutants to agriculture that reflect producer-consumer decision-making processes, associated market adjustments, and some measure of distributional consequences between affected parties. Many of the economic assessments reviewed in previous O_3 documents also focused on O_3 effects in specific regions, primarily California and the Corn Belt (e.g., Garcia et al., 1986). This regional emphasis in the earlier literature may be attributed to the relative abundance of data on crop response and air quality for selected U.S. regions, as well as the importance of some agricultural regions (such as California) for the U.S. agricultural economy.

Two U.S. national studies described in previous O_3 criteria documents that are worthy of additional comment are Kopp et al. (1985) and Adams et al. (1986). These were judged to be "adequate" in terms of the three critical areas of data inputs in the 1996 AQCD. Together, it was argued, they provide a reasonably comprehensive estimate of the economic consequences of

changes in ambient air O_3 levels on agriculture. Because of their central role in the 1996 criteria document, these two studies are reviewed briefly below.

The Kopp et al. (1985) and Adams et al. (1986) studies indicated that ambient levels of O_3 were imposing substantial economic costs of ~$3.4 billion (in 2000 U.S. dollars) on agriculture. Both were judged to suffer from several sources of uncertainty, but the document concluded that these possible improvements in future assessments were not likely to greatly alter the range of agricultural benefit estimates arising from O_3 reductions for several reasons. First, the studies covered about 75 to 80 % of U.S. agricultural crops (by value). For inclusion of the other 20% to significantly change the estimates would require that their sensitivities to O_3 be much greater than for the crops that have been included to date. Second, model-sensitivity analyses reported in past studies indicate that changes in plant exposure-response relationships must be substantial to translate into major changes in economic benefits estimates. For example, it was assumed unlikely that use of different exposure measures, or inclusion of interaction effects, would greatly alter the magnitude of the economic estimates. Third, it was believed that countervailing effects would mitigate against large swings in the estimates, e.g., longer O_3-exposure periods may predict greater yield losses, but O_3-water stress interactions tend to reduce the yield estimates.

Other national assessments reported in the 1996 AQCD provided general corroboration of the results of Kopp et al. (1985) and Adams et al. (1986). An evaluation of these studies, in terms of the adequacy of information from plant exposure studies and aerometric and economic data, was presented in the 1996 AQCD, along with estimates of benefits or damages associated with changes in O_3. Most of the studies added onto either Kopp et al. (1985) or Adams et al. (1986). A relevant question was whether subsequent studies provided any "surprises" in terms of magnitude of economic effects.

Common themes or findings from these and earlier O_3 and other air pollution studies were summarized in two synthesis papers, those of Adams and Crocker (1989) and Segerson (1991). The major conclusion is that the agricultural effects of tropospheric O_3 at ambient levels impose economic costs to society or, conversely, that reductions in ambient O_3 should result in societal benefits.

Several studies contained in the 1996 AQCD still are of interest. For example, one finding pertains to the relationship between federal farm programs and air pollution regulations (McGartland, 1987). In each case, the inclusion of farm programs in the economic models resulted in modest reductions in the economic benefits of O_3 control due to increased farm program costs. As Segerson (1987) noted, however, it is not clear that these increased costs should be charged against the potential benefits of an O_3-regulatory standard but, rather, considered as an additional cost associated with the inefficiencies of the farm program. It should also be noted that the nature of federal farm programs was changed dramatically by Congress in 1996 in an attempt to reduce the federal government's role in agriculture. Although more recent federal legislation, such as the 2002 Farm Bill (U.S. Congress, 2002), appears to be restoring the federal government's role in the farming sector, this issue currently is not as important as suggested by earlier studies, due to the declining reliance on deficiency payments to farmers, which tend to distort resource allocation.

Another national study (Adams et al., 1988) analyzed economic benefits under a regulatory alternative involving a seasonal (crop growing season) O_3-exposure index measured as a 12-h mean, instead of hourly levels or percent changes from ambient as reported in earlier studies. Specifically, a seasonal average of 50 ppb O_3 (measured as a 12-h seasonal mean), with a 95% compliance level, was reported in Adams et al. (1988). The result (a $2.9 billion benefit in 2000 dollars) is similar to the assumed 25% reduction across all regions reported by Adams et al. (1986). At least one study also combined environmental stressors (e.g., O_3, UV-B radiation) in performing economic assessments. Adams and Rowe (1990), using the same model as Adams et al. (1986), reported that a 15% depletion of stratospheric O_3 (resulting in a 13% increase in tropospheric O_3) would cause an economic loss of ~$1.4 billion in 2000 dollars attributed to the tropospheric O_3 increase. Reducing VOCs/NO_x motor vehicle emissions by 10% would result in a benefit of ~$0.3 billion, while a complete elimination of motor vehicle emissions would yield a benefit of ~$3.4 billion (1990 dollars). The range of these numbers is consistent with values reported in Adams et al. (1986), Kopp et al. (1985), and other national-level analyses, i.e., estimates of from $1.0 to 2.0 billion for reductions in ambient O_3 of 25 to 40%.

AX9.7.3.2 Forests (Tree Species) and Natural Ecosystems

The long-term nature of air pollution effects on perennial species such as trees creates challenges to plant scientists in attempts to sort out the effects of specific individual stressors such as O_3 from among the many other potential causal factors (Skelly, 1988). It also creates problems in terms of measuring the impacts on direct economic value of goods, such as reductions in board-feet of lumber produced per unit of time.

Most of the literature in the 1996 AQCD dealing with forest species reported the effects of O_3 exposures in terms of foliar injury (Davis and Skelly, 1992; Freer-Smith and Taylor, 1992; Simini et al., 1992; Taylor and Hanson, 1992). This emphasis on foliar effects in the forest effects literature (rather than marketable yield) is similar to the state of science for agricultural crops prior to 1980. More recent studies address the effects of air pollutants on forest tree species diversity (Bringmark and Bringmark, 1995; Vacek et al., 1999; Weiner et al., 1997). However, such information is of limited use in economic assessments. The exception is in measuring the economic value of aesthetic changes in a forest stock, where changes in species composition may affect recreational values (Crocker, 1985).

The data concerning changes in marketed output, such as board-feet of lumber or changes in growth rates in managed forests or effects on the growth of almond, peach, apricot, pear and plum trees in orchards, cited in the 1996 document (U.S. Environmental Protection Agency, 1996) have not been quantified. In addition, the economic impact of reductions in growth of seedlings of evergreen trees, e.g., slash pine, presented in the same document have not been valued. The few studies which attempted to measure economic losses arising from exposures to O_3 or other pollutants circumvented the lack of plant science data by assuming (often arbitrary) reductions in forest species growth or harvest rates (Adams, 1986; Callaway et al., 1986; Crocker and Forster, 1986; Haynes and Adams, 1992). Although the economic estimates reported in the 1996 AQCD are comparable to those reported for agriculture (e.g., $2.6 billion for eastern Canada forests, $2.9 billion for eastern U.S. forests in 2000 dollars), the lack of yield and/or growth effects data makes these studies only suggestive at best, of the economic consequences of forest effects directly attributable to O_3 exposures questionable. Recent developments in forestry economic modeling capabilities, in support of climate change research, have enhanced the ability to measure the effects of environmental stressors on this sector (Adams et al., 1996; McCarl et al., 1998). However, these models need data on changes in either

timber production or growth rates, both of which are lacking for forest species under alternative O_3 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 O_3 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 O_3 on California crops. Their results showed net benefits from reductions in ambient O_3 levels, a finding consistent with all previous economic assessments. In another study, Westenbarger and Frisvold (1994) measured agricultural exposures to O_3 (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 O_3 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 O_3 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 O_3 exposures from all sources, i.e., ambient O_3 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 O_3 levels. The study is also notable in its careful attention to federal farm program effects, particularly the deficiency payment component.

In addition to these studies in peer-reviewed journals, a number of site-specific effects studies have been performed, primarily by consulting companies for state public utility commissions. Although perhaps of use to public utility commissioners concerned with effects from single power plants or other localized sources, these regional studies generally contribute little to the assessment of air pollution effects at the national level. Also, such reports are not peer reviewed. Therefore, they are not discussed here.

There have been a number of recent studies of air pollutant effects on tree species in the literature. Some have reported changes in total biomass and focused on European species (Kurczynska et al., 1997). Other studies have assessed changes in composition of forest species (biodiversity) or forest health due to exposure to air pollutants (Bringmark and Bringmark, 1995; McLaughlin and Percy, 1999; Vacek et al., 1999). As noted previously, changes in forest biomass and composition are more difficult to value than marketable products. However, measures of forest composition or health have implications for an area of increasing policy concern, that being the effect of air pollutants and other environmental stressors on unmanaged (natural) ecosystems and the services they provide (Goulder and Kennedy, 1997; Pimentel et al., 1997). Considerable discussion has occurred among ecologists and economists as to the appropriate means for valuing these services (Anderson, 1990; Carpenter and Dixon, 1985; Common and Perrings, 1992). A number of conceptual articles have been published on this issue in both economic and ecological journals (Bergstrom, 1990; Castle, 1993; Pearce, 1993; Suter, 1990).

A continuing empirical challenge concerns the lack of information on how changes in biodiversity affect ecosystem performance resulting from O_3 stresses and the problem of establishing economic values for such changes (Cairnes and Lackey, 1992; Norton, 1987; Pimm, 1997; Polasky, 2001; Randall, 1991). As noted in the 1996 AQCD, and more recently by Daily (1999, 2000) and Polasky (2001), there continues to be a lack of empirical studies that actually assess the economic value of changes in biodiversity or in service flows due to any environmental stressors. While some studies report monetary estimates, the estimates are generally for expository purposes and those would not be as defensible as the agricultural studies described earlier. For example, Costanza et al. (1997) assigned a value to the world's ecosystems, but the procedures used render this an exploratory study at best. As assessed by Polasky (2001), "In general, the field of valuation of ecosystem services is in its infancy."

He attributed the lack of empirical studies due to both a lack of the understanding of ecology of ecosystem services and to the absence of reliable methods to estimate the value of these services.

In summary, the studies of crop and forest responses in the economic literature indicate that O_3 reduces crop yields and imposes economic costs. The economic literature also indicates that O_3 adversely influences the physiological performance of tree species and demonstrates, as expected, that changes in growth have economic consequences. However, the economic data and literature available on ecosystem effects of O_3 exposures are not sufficient to determine the economic costs.

AX9.7.5 Limitations of Scientific Studies and Economic Information

The 1996 O_3 AQCD discussed the need for additional research on both ecological functions and economic methodology in order to better understand the economic implications of air pollutants on ecosystem services. As noted by Daily (1999, 2000), Polasky (2001), and others, this research agenda continues to be important. Despite the large number of discussion and survey articles published since 1996, there still are not sufficient data by which to estimate confidently the magnitude of economic effects of O_3 to forests and natural ecosystems. Nor is it apparent that ongoing research is adequate to answer this question in the next criteria document cycle. Specifically, there do not appear to be any comprehensive, ecological studies underway that attempt to measure changes in ecosystem outputs under alternative O_3 or other air pollutant levels. Thus, in the near term, ecosystem services can only be discussed in qualitative terms. However plausible the likelihood of economic damages to ecosystems, the available scientific and economic information does not provide specific guidance on the magnitudes of these effects.

Beside the need to improve our understanding of the effects of O_3 exposures on natural ecosystems, a number of areas of research could help assess the full economic consequences of such pollutants on managed ecosystems. The first of these is the relationship between O_3 exposure levels and the variability of crop yields or changes in forest biodiversity. Most assessments are based on the average or expected yield response of a crop to air pollution exposure. However, the variability in yields (the spread or dispersion around the mean) appears to be affected by the nature of plant exposure to pollutants (Hogsett et al., 1985a; Musselman et al., 1983). Plants exposed to the same mean dose but with different second moments of the distribution of exposure may have different mean yields (Altshuller and Lefohn, 1996; Lefohn

and Benedict, 1982). In addition, the variability of the yield of the plant may also be increased by greater variability in exposures (e.g., a higher frequency of extreme events). The economic significance of higher yield varieties is such that variability may impose additional economic costs, because most farmers have been reported to be averse to risk and prefer less variability for a given yield (or profit). To date, no economic assessments of O_3 damages to agriculture or vegetation in general include risk-averse behavior (studies cited here assumed risk neutrality). To assess the economic consequences of a relationship between farmers' risk preferences and O_3-induced changes in yield variability will require more information on the potential effects of changes in O_3 on crop yield variability. While no economic studies were found on the effects of O_3-induced changes on yield variability, a number of recent studies of climate change effects on crop yields have indicated increased economic costs in the presence of increased climatic variability (e.g., Mearns et al., 1997; Dixon and Segerson, 1999). Analogous economic costs would be expected for changes in air pollution distributions; and these effects need to be examined and quantified.

Another research area concerns the need improvement in our understanding of temporal (dynamic) and spatial characteristics of O_3 exposures and their implications for crop yields, production and producer profits. Most economic studies are static, in that they compare two states of the world (e.g., economic activity at one O_3 level versus at an alternative level). In addition, most national-level studies (the type needed to evaluate Secondary National Ambient Air Quality Standards [SNAAQS]) display coarse regional-level resolution in terms of crop response, O_3 exposure, and economic behavior. The responses of producers to changes in yields due to changes in O_3 levels are generally assumed to be similar over geographical areas up to several states in size. However, the changes between air quality scenarios are more likely to be characterized by transient changes in exposure levels, which means the producer responses are also likely to be gradual, rather than abrupt. Similarly, the lack of finer scale (regional-level) data and modeling capabilities suggests that important micro-level physical and economic effects are ignored. To what extent these abstractions influence net economic effects is an empirical question. Research on these types of abstractions and assumptions within other economic settings, such as climatic change, have shown that they have implications for economic measurements (Adams, 2002).

Another issue is the natural or background level of O_3 (or other pollutants of interest) assumed in economic studies. While many economic studies focus on changes in pollution levels from current conditions, some studies have measured the economic damages between an assumed, or pristine, level and current levels in agricultural areas. Such an analysis, it is reasoned, will provide a measure of the net damages due to anthropogenic sources. The challenge here is to have a correct measure of the background level of the pollutant. Recent research by Lefohn et al. (2001) has suggested that background levels may be considerably higher than assumed in some of the previous economic assessments reported in the 1996 AQCD (25 to 30 ppb in most studies). For example, Lefohn et al. (Lefohn et al., 2001) detected hourly readings of from 40 to 80 ppb during winter and spring months in remote areas of the United States. If background O_3 levels are in the range, then the economic damages estimated in studies with lower background levels will be overstated. The issue of the background O_3 level is important to all assessments of vegetative damages due to O_3.

In terms of expanding economic methods for future assessment, analysts should consider using more "reduced form" estimation methods, particularly in situations where the availability of dose-response functions is limited. This estimation approach is exemplified by Garcia et al. (1986). Specifically, their econometric study of the impact of O_3 on producer profits used such a reduced-form approach. In this approach, farmer actions are modeled as a function of ambient O_3 levels (along with other explanatory variables) without the direct use of dose response functions. The advantage of this procedure is that one source of modeling uncertainty, the need for dose-response functions, including time-consuming crop experiments to generate data, is reduced. Actual responses of farmers' profits across air pollution gradients of ambient pollution are observed instead of hypothesized. Although this procedure has not been widely used in air pollution economic assessments, it has been used in a number of relatively recent climate change studies (e.g., Mendelsohn et al., 1994). The reduced-form method suffers from the fact that if proposed O_3 levels are lower than those observed in the estimation sample, then the prediction accuracy of the method deteriorates. Also, some dose-response information is needed, if only to establish the plausibility of the economic estimates.

Another area that may improve economic assessments is incorporating consideration of livestock issues. To date, most agricultural economic assessments of O_3 impacts have ignored the livestock sector. Presumably this is because ambient O_3 levels do not noticeably affect meat

yields. However, if feed prices and pasture conditions are affected by ambient O_3 levels, then a more accurate estimate of economic impacts would be forthcoming by including this link to livestock in the assessment. These types of feed production and feed price effects are included in the mathematical programming model used in Adams et al. (1986), but not in most other O_3 effects assessments. The significance of livestock-feed linkages are demonstrated in a recent study in the Netherlands by Kuik et al (2000). Using a mathematical programming model similar to that in Adams et al. (1986), Kuik et al. (2000) found that livestock effects were prominent, due mainly to improved pasture yields under reduced ambient O_3 levels.

AX9.7.6 Conclusions

Substantial progress has been made over the past two decades in our understanding of the effects of O_3 and other oxidants on vegetation, particularly for agriculturally important plant species. The physical and economic effects on agriculture are well documented and provide useful information for the setting of SNAAQS. Effects on forests and natural ecosystems remain problematic, due to limitations in natural science data and economic methods. The problem is most acute for valuing natural ecosystem service flows.

The current limitations surrounding forests and natural ecosystems present a rich research agenda. However, not all research needs are likely to lead to better policies. Thus, areas of greatest potential value in terms of regional policymaking need to be prioritized. Such priority setting can be assisted by sensitivity analyses with existing economic models. By measuring the changes in economic effects arising from changes in key parameters, research data gaps most likely to affect economic values can be identified.

REFERENCES

Abeles, F. B.; Biles, C. L.; Dunn, L. J. (1989a) Hormonal regulation and distribution of peroxidase isoenzymes in the cucurbitaceae. Plant Physiol. 91: 1609-1612.

Abeles, F. B.; Hershberger, W. L.; Dunn, L. J. (1989b) Hormonal regulation and intracellular localization of a 33 kda cationic peroxidase in excised cucumber cotyledons. Plant Physiol. 89: 664-668.

Adams, R. M. (1986) Agriculture, forestry, and related benefits of air pollution control: a review and some observations. Am. J. Agric. Econ. 68: 464-472.

Adams, R. M. (1999) On the search for the correct economic assessment method: an editorial comment. Clim. Change 41: 363-370.

Adams, W. C. (2002) Comparison of chamber and face-mask 66-hour exposures to ozone on pulmonary function and symptoms responses. Inhalation Toxicol. 14: 745-764.

Adams, R. M.; Crocker, T. D. (1989) The agricultural economics of environmental change: some lessons from air pollution. J. Environ. Manage. 28: 295-307.

Adams, R. M.; Horst, R. L., Jr. (2003) Future directions in air quality research economic issues. Environ. Int. 29: 289-302.

Adams, M. B.; O'Neill, E. G. (1991) Effects of ozone and acidic deposition on carbon allocation and mycorrhizal colonization of *Pinus taeda* L seedlings. For. Sci. 37: 5-16.

Adams, R. M.; Rowe, R. D. (1990) The economic effects of stratospheric ozone depletion on US agriculture: a preliminary assessment. J. Environ. Manage. 30: 321-335.

Adams, R. M.; Hamilton, S. A.; McCarl, B. A. (1985) An assessment of the economic effects of ozone on U.S. agriculture. J. Air Pollut. Control Assoc. 35: 938-943.

Adams, R. M.; Hamilton, S. A.; McCarl, B. A. (1986) The benefits of pollution control: the case of ozone and U.S. agriculture. Am. J. Agric. Econ. 68: 886-893.

Adams, R. M.; Glyer, J. D.; McCarl, B. A. (1988) The NCLAN economic assessment: approach, findings and implications. In: Heck, W. W.; Taylor, O. C.; Tingey, D. T., eds. Assessment of crop loss from air pollutants: proceedings of an international conference; October 1987; Raleigh, NC. New York, NY: Elsevier Applied Science; pp. 473-504.

Adams, D. M.; Alig, R. J.; Callaway, J. M.; McCarl, B. A.; Winnett, S. M. (1996) The forest and agricultural sector optimization model (FASOM): model structure and policy application. Portland, OR: U.S. Department of Agriculture, Pacific Northwest Research Station; PNW-RP-495.

Adams, R. M.; Hurd, B. H.; Lenhart, S.; Leary, N. (1998) Effects of global climate change on agriculture: an interpretative review. Clim. Res. 11: 19-30.

Agrawal, M.; Agrawal, S. B. (2000) Effects of air pollution on plant diversity. In: Agrawal, S. B.; Agrawal, M., eds. Environmental Pollution and Plant Responses. Lewis Publishers; pp. 137-152.

Ainsworth, E. A.; Long, S. P. (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol. 165: 351-371.

Ainsworth, N.; Fumagalli, I.; Giorcelli, A.; Mignanego, L.; Schenoe, G.; Vietto, L. (1996) Assessment of EDU stem injections as a technique to investigate the response of trees to ambient ozone in field conditions. Agric. Ecosyst. Environ. 59: 33-42.

Ainsworth, E. A.; Davey, P. A.; Bernacchi, C. J.; Dermody, O. C.; Heaton, E. A.; Moore, D. J.; Morgan, P. B.; Naidu, S. L.; Yoora, H. S.; Zhu, X. G.; Curtis, P. S.; Long, S. P. (2002) A meta-analysis of elevated [CO_2] effects on soybean (*Glycine max*) physiology, growth and yield. Global Change Biol. 8: 695-709.

Alexis, A.; Garcia, A.; Nystrom, M.; Rosenkranz, K. (2001) The 2001 California almanac of emissions and air quality. Sacramento, CA: California Air Resources Board, California Planning and Technical Support Division. Available: http://www. arb. ca. gov/aqd/almanac/almanac01/almanac01. htm [10 August, 2005].

Alscher, R. G.; Wellburn, A. R. (1994) Plant responses to the gaseous environment. Molecular, metabolic and physiological aspects. London, UK: Chapman and Hall.

Alscher, R. G.; Donahue, J. L.; Cramer, C. L. (1997) Reactive oxygen species and antioxidants: relationships in green cells. Physiol. Plant. 100: 224-233.

Altimir, N.; Vesala, T.; Keronen, P.; Kulmala, M.; Hari, P. (2002) Methodology for direct field measurements of ozone flux to foliage with shoot chambers. Atmos. Environ. 36: 19-29.

Altimir, N.; Tuovinen, J. P.; Vesala, T.; Kulmala, M.; Hari, P. (2004) Measurements of ozone removal by Scots pine shoots: calibration of a stomatal uptake model including the non-stomatal component. Atmos. Environ. 38: 2387-2398.

Altshuller, A. P.; Lefohn, A. S. (1996) Background ozone in the planetary boundary layer over the United States. J. Air Waste Manage. Assoc. 46: 134-141.
Alvarado, D.; De Bauer, L. I.; Galindo, J. (1993) Decline of sacred fir (*Abies religiosa*) in a forest park south of Mexico City. Environ. Pollut. 80: 115-121.
Amiro, B. D.; Gillespie, T. J. (1985) Leaf conductance response of *Phaseolus vulgaris* to ozone flux density. Atmos. Environ. 19: 807-810.
Amiro, B. D.; Gillespie, T. J.; Thurtell, G. W. (1984) Injury response of *Phaseolus vulgaris* to ozone flux density. Atmos. Environ. 18: 1207-1215.
Amthor, J. S.; Goulden, M. L.; Munger, J. W.; Wofsy, S. C. (1994) Testing a mechanistic model of forest-canopy mass and energy exchange using eddy correlation: carbon dioxide uptake and ozone uptake by a mixed oak-maple stand. Aust. J. Plant Physiol. 211: 623-651.
Anderson, E. (1990) The ethical limitations of the market. Econ. Philos. 6: 179-205.
Andersen, C. P. (2000) Ozone stress and changes below-ground: linking root and soil processes. Phyton 40(Special issue 4): 7-12.
Andersen, C. P. (2003) Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol. 157: 213-228.
Andersen, C. P.; Rygiewicz, P. T. (1991) Stress interactions and mycorrhizal plant response: understanding carbon allocation priorities. Environ. Pollut. 73: 217-244.
Andersen, C. P.; Rygiewicz, P. T. (1995a) Allocation of carbon in mycorrhizal *Pinus ponderosa* seedlings exposed to ozone. New Phytol. 131: 471-480.
Andersen, C. P.; Rygiewicz, P. T. (1995b) Effect of ozone on temporal allocation of carbon in mycorrhizal of *Pinus ponderosa* seedlings. New Phytol. 131: 471-480.
Andersen, C. P.; Scagel, C. F. (1997) Nutrient availability alters belowground respiration of ozone-exposed ponderosa pine. Tree Physiol. 17: 377-387.
Anderson, R. L.; Brown, H. D.; Chevone, B. I.; McCartney, T. C. (1988) Occurence of air pollution symptoms (needle tip necrosis and chlorotic mottling) on eastern white pine in the southern Appalachian Mountains. Plant Dis. 72: 130-132.
Andersen, C. P.; Hogsett, W. E.; Wessling, R.; Plocher, M. (1991) Ozone decreases spring root growth and root carbohydrate content in ponderosa pine the year following exposure. Can. J. For. Res. 21: 1288-1291.
Andersen, C. P.; Wilson, R.; Plocher, M.; Hogsett, W. E. (1997) Carry-over effects of ozone on root growth and carbohydrate concentrations of ponderosa pine seedlings. Tree Physiol. 17: 805-811.
Andersen, C. P.; Hogsett, W. E.; Plocher, M.; Rodecap, K.; Lee, E. H. (2001) Blue wild-rye grass competition increases the effect of ozone on ponderosa pine seedlings. Tree Physiol. 21: 319-327.
Antonielli, M.; Pasqualini, S.; Ederli, L.; Batini, P.; Moscatello, S.; Loreto, F. (1997) Physiological characteristics of tobacco cultivars with contrasting sensitivity to ozone. Environ. Exp. Bot. 38: 271-277.
Anttonen, S.; Sutinen, M. L.; Heagle, A. S. (1996) Ultrastructure and some plasma membrane characteristics of ozone-exposed loblolly pine needles. Physiol. Plant. 98: 309-319.
Aono, M.; Saji, H.; Fujiyama, K.; Sugita, M.; Kondo, N.; Tanaka, K. (1995) Decrease in activity of glutathione reductase enhances paraquat sensitivity in transgenic *nicotiana tabacum*. Plant Physiol. 107: 645-648.
Arbaugh, M. J.; Miller, P. R.; Carroll, J. J.; Takemoto, B. L.; Proctor, T. (1998) Relationships of ozone exposure to pine injury in the Sierra Nevada and San Bernardino Mountains of California, USA. Environ. Pollut. 101: 291-301.
Arbaugh, M. J.; Johnson, D. W.; Pulliam, W. M. (1999) Simulated effects of N deposition, ozone injury, and climate change on a forest stand in the San Bernardino Mountains. In: Miller, P. R.; McBride, J. R., eds. Oxidant air pollution impacts in the montane forests of southern California. New York, NY: Springer (Ecological Studies, Vol. 134); pp. 179-207.
Arbaugh, M.; Bytnerowicz, A.; Grulke, N.; Fenn, M.; Poth, M.; Temple, P.; Miller, P. (2003) Photochemical smog effects in mixed conifer forests along a natural gradient of ozone and nitrogen deposition in the San Bernardino Mountains. Environ. Int. 29: 401-406.
Arndt, U. (1995) Air pollutants and pheromones - a problem? Chemosphere 30: 1023-1031.
Arp, W. J.; Drake, B. G. (1991) Increased photosynthetic capacity of *Scirpus olneyi* after 4 years of exposure to elevated CO_2. Plant Cell Environ. 14: 1003-1006.
Asada, K. (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Ann. Rev. Plant Physiol. Mol. Biol. 50: 601-639.
Ashenden, T. W.; Bell, S. A.; Rafarel, C. R. (1995) Responses of white clover to gaseous pollutants and acid mist: implications for setting critical levels and loads. New Phytol. 130: 89-96.

Ashenden, T. W.; Bell, S. A.; Rafarel, C. R. (1996) Interactive effects of gaseous air pollutants and acid mist on two major pasture grasses. Agric. Ecosyst. Environ. 57: 1-8.
Ashmore, M. R.; Ainsworth, N. (1995) The effects of ozone and cutting on the species composition of artificial grassland communities. Funct. Ecol. 9: 708-712.
Ashmore, M. R.; Bell, J. N. B.; Mimmack, A. (1988) Crop growth along a gradient of ambient air pollution. Environ. Pollut. 53: 99-121.
Ashmore, M. R.; Thwaites, R. H.; Ainsworth, N.; Cousins, D. A.; Power, S. A.; Morton, A. J. (1995) Effects of ozone on calcareous grassland communities. Water Air Soil Pollut. 85: 1527-1532.
Ashmore, M.; Emberson, L.; Karlsson, P. E.; Pleijel, H. (2004a) New directions: a new generation of ozone critical levels for the protection of vegetation in Europe (correspondence). Atmos. Environ. 38: 2213-2214.
Ashmore, M. E.; Karlsson, P. E.; Pleijel, H. (2004b) Introduction for ozone deposition special issue. Atmos. Environ. 38: 2211-2212.
Assmann, S. M. (2003) Open stomata 1 opens the door to ABA signaling in *Arabidopsis* guard cells. Trends Plant Sci. 8: 151-153.
Assmann, S. M.; Wang, X. Q. (2001) From milliseconds to millions of years: guard cells and environmental responses. Curr. Opin. Plant Biol. 4: 421-428.
Atkinson, R. (1990) Gas-phase tropospheric chemistry of organic compounds: a review. Atmos. Environ. Part A 24: 1-41.
Auh, C. K.; Murphy, T. M. (1995) Plasma membrane redox enzyme is involved in the synthesis of O_2^- and H_2O_2 by *phytophthora* elicitor-stimulated rose cells. Plant Physiol. 107: 1241-1247.
Awmack, C. S.; Harrington, R.; Lindroth, R. L. (2004) Aphid individual performance may not predict population responses to elevated CO_2 or O_3. Global Change Biol. 10: 1414-1423.
Ayres, P. G. (1991) Growth responses induced by pathogens and other stresses. In: Mooney, H. A.; Winner, W. E.; Pell, E. J., eds. Responses of plants to multiple stresses. San Diego, CA: Academic Press; pp. 227-248.
Azevedo, R. A.; Alas, R. M.; Smith, R. J.; Lea, P. J. (1998) Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiol. Plant. 104: 280-292.
Bäck, J.; Vanderklein, D. W.; Topa, M. A. (1999) Effects of elevated ozone on CO_2 uptake and leaf structure in sugar maple under two light environments. Plant Cell Environ. 22: 137-147.
Bahl, A.; Kahl, G. (1995) Air pollutant stress changes the steady-state transcript levels of three photosynthesis genes. Environ. Pollut. 88: 57-65.
Bahl, A.; Loitsch, S. M.; Kahl, G. (1995) Transcriptional activation of plant defence genes by short-term air pollutant stress. Environ. Pollut. 89: 221-227.
Baker, T. R.; Allen, H. L.; Schoeneberger, M. M.; Kress, L. W. (1994) Nutritional response of loblolly pine exposed to ozone and simulated acid rain. Can. J. For. Res. 24: 453-461.
Baker, C. J.; Harmon, G. L.; Glazener, J. A.; Orlandi, E. W. (1995) A noninvasive technique for monitoring peroxidative and H2O2-scavenging activities during interactions between bacterial plant pathogens and suspension cells. Plant Physiol. 108: 353-359.
Balaguer, L.; Barnes, J. D.; Panicucci, A.; Borland, A. M. (1995) Production and utilization of assimilates in wheat (*Triticum aestivum* L) leaves exposed to elevated O_3 and/or CO_2. New Phytol. 129: 557-568.
Ball, J. T. (1987) Calculations related to gas exchange. In: Zeiger, E.; Farquhar, G. D.; Cowan, I. R. S.; pp. 445-476, eds. ; Farquhar, G. Stanford Press; pp. 445-476.; pp. G-47.
Ball, G. R.; Benton, J.; Palmer-Brown, D.; Fuhrer, J.; Skärby, L.; Gimeno, B. S.; Mills, G. (1998) Identifying factors which modify the effects of ambient ozone on white clover (*Trifolium repens*) in Europe. Environ. Pollut. 103: 7-16.
Ball, G. R.; Palmer-Brown, D.; Fuhrer, J.; Skärby, L.; Gimeno, B. S.; Mills, G. (2000) Identification of non-linear influences on the seasonal ozone dose-response of sensitive and resistant clover clones using artificial neural networks. Ecol. Model. 129: 153-168.
Balls, G. R.; Palmer-Brown, D.; Cobb, A. H.; Sanders, G. E. (1995) Towards unravelling the complex interactions between microclimate, ozone dose, and ozone injury in clover. Water Air Soil. Pollut. 85: 1467-1472.
Balls, G. R.; Palmer-Brown, D.; Sanders, G. E. (1996) Investigating microclimatic influences on ozone injury in clover (*Trifolium subterraneum*) using artificial neural networks. New Phytol. 132: 271-280.
Banerjee, S. K.; Mudd, J. B. (1992) Reaction of ozone with glycophorin in solution and in lipid vesicles. Arch. Biochem. Biophys. 295: 84-89.
Barbo, D. N.; Chappelka, A. H.; Somers, G. L.; Miller-Goodman, M. S.; Stolte, K. (1998) Diversity of an early successional plant community as influenced by ozone. New Phytol. 138: 653-662.

Barbo, D. N.; Chappelka, A. H.; Somers, G. L.; Miller-Goodman, M. S.; Stolte, K. (2002) Ozone impacts on loblolly pine (*Pinus taeda* L) grown in a competitive environment. Environ. Pollut. 116: 27-36.

Barnes, J. D.; Pfirrmann, T. (1992) The influence of CO_2 and O_3, singly and in combination, on gas exchange, growth and nutrient status of radish (*Raphanus sativus* L). New Phytol. 121: 403-412.

Barnes, J. D.; Wellburn, A. R. (1998) Air pollutant combinations. In: De Kok, L. J.; Stulen, I., eds. Responses of plant metabolism to air pollution and global change. Leiden, The Netherlands: Backhuys Publishers; pp. 147-164.

Barnes, J. D.; Ollerenshaw, J. H.; Whitfield, C. P. (1995) Effects of elevated CO_2 and/or O_3 on growth, development and physiology of wheat (*Triticum aestivum* L). Global Change Biol. 1: 129-142.

Barnes, J.; Zheng, Y.; Lyons, T. (2002) Plant resistance to ozone: the role of ascorbate. In: Omasa, K.; Saji, H.; Youssefian, S.; Kondo, N., eds. Air pollution and plant biotechnology - prospects for phytomonitoring and phytoremediation. Air pollution and plant biotechnology - prospects for phytomonitoring and phytoremediation. Springer-Verlag; pp. 235-252.

Barrett, S. C. H.; Bush, E. J. (1991) Population processes in plants and the evolution of resistance to gaseous air pollutants. In: Taylor, G. E., Jr.; Pitelka, L. F.; Clegg, M. T., eds. Ecological genetics and air pollution. Springer-Verlag; pp. 137-165.

Bartholomay, G. A.; Eckert, R. T.; Smith, K. T. (1997) Reductions in tree-ring widths of white pine following ozone exposure at Acadia National Park, Maine, USA. Can. J. For. Res. 27: 361-368.

Bassin, S.; Calanca, P.; Weidinger, T.; Gerosa, G.; Fuhrer, J. (2004) Modeling seasonal ozone fluxes to grassland and wheat: model improvement, testing, and application. Atmos. Environ. 38: 2349-2359.

Batts, G. R.; Morison, J. I. L.; Ellis, R. H.; Hadley, P.; Wheeler, T. R. (1997) Effects of CO_2 and temperature on growth and yield of crops of winter wheat over four seasons. Eur. J. Agron. 7: 43-52.

Bauer, M. R.; Hultman, N. E.; Panek, J. A.; Goldstein, A. H. (2000) Ozone deposition to a ponderosa pine plantation in the Sierra Nevada Mountains (CA): a comparison of two different climatic years. J. Geophys. Res. [Atmos.] 105: 22,123-22,136.

Baumbusch, L. O.; Eiblmeier, M.; Schnitzler, J. P.; Heller, W.; Sandermann, H., Jr.; Polle, A. (1998) Interactive effects of ozone and low UV-B radiation on antioxidants in spruce (*Picea abies*) and pine (*Pinus sylvestris*). Physiol. Plant. 104: 248-254.

Baumgardner, R. E.; Edgerton, E. S. (1998) Rural ozone across the eastern United States: analysis of CASTNet data, 1988-1995. J. Air Waste Manage. Assoc. 48: 674-688.

Baumgarten, M.; Werner, H.; Häberle, K. H.; Emberson, L. D.; Fabian, P.; Matyssek, R. (2000) Seasonal ozone response of mature beech trees (*Fagus sylvatica*) at high altitude in the Bavarian Forest (Germany) in comparison with young beech grown in the field and in phytotrons. Environ. Pollut. 109: 431-442.

Beare, J. A.; Archer, S. A.; Bell, J. N. B. (1999a) Effects of *Melampsora* leaf rust disease and chronic ozone exposure on poplar. Environ. Pollut. 105: 419-426.

Beare, J. A.; Archer, S. A.; Bell, J. N. B. (1999b) *Marssonina* leafspot disease of poplar under elevated ozone: pre-fumigated host and in vitro studies. Environ. Pollut. 105: 409-417.

Beligni, M. V.; Lamattina, L. (2001) Nitric oxide in plants: the history is just beginning. Plant Cell Environ. 24: 267-278.

Beligni, M.; Lamattina, L. (2002) Nitric oxide interferes with plant photo-oxidative stress by detoxifying reactive oxygen species. Plant Cell Environ. 25: 737-748.

Bell, J. N. B.; Treshow, M. (2002) Air pollution and plant life. Chichester, UK: John Wiley & Sons Ltd., 465.

Bell, J. N. B.; Ashmore, M. R.; Wilson, G. B. (1991) Ecological genetics and chemical modifications of the atmosphere. In: Taylor, G. E., Jr.; Pitelka, L. F.; Clegg, M. T., eds. Ecological genetics and air pollution. Springer-Verlag; pp. 33-59.

Bender, J.; Weigel, H. J. (1992) Crop responses to mixtures of air pollutants. In: Jäger, H. J.; Unsworth, M.; De Temmerman, L.; Mahy, P., eds. Effects of air pollution on agricultural crops in Europe. Commission of the European Communities; pp. 445-454.

Bender, J.; Weigel, H. J.; Jager, H. J. (1991) Response of nitrogen metabolism in beans (*Phaseolus vulgaris* L) after exposure to ozone and nitrogen dioxide, alone and in sequence. New Phytol. 119: 261-267.

Bender, J.; Weigel, H. J.; Wegner, U.; Jäger, H. J. (1994) Response of cellular antioxidants to ozone in wheat flag leaves at different stages of plant development. Environ. Pollut. 84: 15-21.

Bender, J.; Herstein, U.; Black, C. R. (1999) Growth and yield responses of spring wheat to increasing carbon dioxide, ozone and physiological stresses: a statistical analysis of 'ESPACE-wheat' results. Eur. J. Agron. 10: 185-195.

Benes, S. E.; Murphy, T. M.; Anderson, P. D.; Houpis, J. L. J. (1995) Relationship of antioxidant enzymes to ozone tolerance in branches of mature ponderosa pine (*Pinus ponderosa*) trees exposed to long term, low concentration, ozone fumigation and acid precipitation. Physiol. Plant. 94: 124-134.

Bennett, J. H. (1979) Foliar exchange of gases. In: Heck, W. W.; Krupa, S. V.; Linzon, S. N. eds. Methodology for the assessment of air pollution effects on vegetation: a handbook from a specialty conference; April 1978; Minneapolis, MN; Pittsburgh, PA: Air Pollution Control Association; pp. 10-1-10-29.

Bennett, J. P.; Anderson, R. L.; Mielke, M. L.; Ebersole, J. J. (1994) Foliar injury air pollution surveys of eastern white-pine (*Pinus strobus* L) - a review. Environ. Monit. Assess. 30: 247-274.

Benoit, L. F.; Skelly, J. M.; Moore, L. D.; Dochinger, L. S. (1982) Radial growth reductions of *Pinus strobus* L correlated with foliar ozone sensitivity as an indicator of ozone-induced losses in eastern forests. Can. J. For. Res. 12: 673-678.

Benoit, L. F.; Skelly, J. M.; Moore, L. D.; Dochinger, L. S. (1983) The influence of ozone on *Pinus strobus* L pollen germination. Can. J. For. Res. 13: 184-187.

Benton, J.; Fuhrer, J.; Skärby, L.; Sanders, G. (1995) Results from the UN/ECE ICP-crops indicate the extent of exceedance of the critical levels of ozone in Europe. Water Air Soil Pollut. 85: 1473-1478.

Benton, J.; Fuhrer, J.; Gimeno, B. S.; Skarby, L.; Palmer-Brown, D.; Ball, G.; Roadknight, C.; Sanders-Mills, G. (1996) The critical level of ozone for visible injury on crops and natural vegetation (ICP-Crops). In: Kärenlampi, L.; Skärby, L. eds. Critical levels for ozone in Europe: testing and finalizing the concepts: UN-ECE workshop report. UN-ECE Convention on Long-Range Transboundary Air Pollution; April; Kuopio, Finland; Kuopio, Finland: University of Kuopio, Department of Ecology and Environmental Science; pp. 44-57.

Benton, J.; Fuhrer, J.; Gimeno, B. S.; Skärby, L.; Palmer-Brown, D.; Ball, G.; Roadknight, C.; Mills, G. (2000) An international cooperative programme indicates the widespread occurrence of ozone injury on crops. Agric. Ecosyst. Environ. 78: 19-30.

Bergmann, E.; Bender, J.; Weigel, H.-J. (1999) Ozone threshold doses and exposure-response relationships for the development of ozone injury symptoms in wild plant species. New Phytol. 144: 423-435.

Bergstrom, J. C. (1990) Concepts and measures of the economic value of environmental quality: a review. J. Environ. Manage. 31: 215-228.

Bergweiler, C. J.; Manning, W. J. (1999) Inhibition of flowering and reproductive success in spreading dogbane (*Apocynum androsaemifolium*) by exposure to ambient ozone. Environ. Pollut. 105: 333-339.

Bermejo, V.; Gimeno, B. S.; Granados, I.; Santamaria, J.; Irigoyen, J. J.; Bermajo, R.; Porcuna, J. L.; Mills, G. (2002) Investigating indices to explain the impacts of ozone on the biomass of white clover (*Trifolium repens* L. cv. Regal) at island and coastal sites in Spain. New Phytol. 156: 43-55.

Berrang, P.; Karnosky, D. F.; Mickler, R. A.; Bennett, J. P. (1986) Natural selection for ozone tolerance in *Populus tremuloides*. Can. J. For. Res. 16: 1214-1216.

Berrang, P.; Karnosky, D. F.; Bennett, J. P. (1989) Natural selection for ozone tolerance in *Populus tremuloides*: field verification. Can. J. For. Res. 19: 519-522.

Berrang, P.; Karnosky, D. F.; Bennett, J. P. (1991) Natural selection for ozone tolerance in *Populus tremuloides*: an evaluation of nationwide trends. Can. J. For. Res. 21: 1091-1097.

Bertrand, A.; Robitaille, G.; Nadeau, P.; Castonguay, Y. (1999) Influence of ozone on cold acclimation in sugar maple seedlings. Tree Physiol. 19: 527-534.

Beyers, J. L.; Riechers, G. H.; Temple, P. J. (1992) Effects of long-term ozone exposure and drought on the photosynthetic capacity of ponderosa pine (*Pinus ponderosa* Laws). New Phytol. 122: 81-90.

Bichele, I.; Moldau, H.; Padu, E. (2000) Estimation of plasmalemma conductivity to ascorbic acid in intact leaves exposed to ozone. Physiol. Plant. 108: 405-412.

Bidartondo, M. I.; Ek, H.; Wallander, H.; Soderstrom, B. (2001) Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi? New Phytol. 151: 543-550.

Bielenberg, D. G.; Lynch, J. P.; Pell, E. J. (2001) A decline in nitrogen availability affects plant responses to ozone. New Phytol. 151: 413-425.

Binkley, D. (1992) Sensitivity of forest soils in the western US to acidic deposition. In: Olson, R. K.; Binkley, D.; Böhm, M., eds. The Response of Western Forests to Air Pollution. Springer-Verlag; pp. 153-182.

Birecka, H.; Catalfamo, J.; Urban, P. (1976) Cell isoperoxidases in sweet potato plants in relation to mechanical injury and ethylene. Plant Physiol. 57: 74-79.

Björkman, O.; Demmig-Adams, B. (1995) Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. In: Schulze, E.-D.; Caldwell, M. M., eds. Ecophysiology of photosynthesis. New York, NY: Springer-Verlag; pp. 17-47.

Black, V. J.; Black, C. R.; Roberts, J. A.; Stewart, C. A. (2000) Impact of ozone on the reproductive development of plants. New Phytol. 147: 421-447.
Blankenship, E. E.; Stefanski, L. A. (2001) Statistical estimation of ozone exposure metrics. Atmos. Environ. 35: 4499-4510.
Boerner, R. E. J.; Rebbeck, J. (1995) Decomposition and nitrogen release from leaves of three hardwood species grown under elevated O_3 and/or CO_2. Plant Soil 170: 149-157.
Böhm, M.; McCune, B.; Vandetta, T. (1995) Ozone regimes in or near forests of the western United States: I regional patterns. J. Air Waste Manage. Assoc. 45: 235-246.
Bonello, P.; Heller, W.; Sandermann, H., Jr. (1993) Ozone effects on root-disease susceptibility and defence responses in mycorrhizal and non-mycorrhizal seedlings of Scots pine (*Pinus sylvestris* L). New Phytol. 124: 653-663.
Booker, F. L. (2000) Influence of carbon dioxide enrichment, ozone and nitrogen fertilization on cotton (*Gossypium hirsutum* L) leaf and root composition. Plant Cell Environ. 23: 573-583.
Booker, F. L.; Miller, J. E. (1998) Phenylpropanoid metabolism and phenolic composition of soybean [*Glycine max* (L) Merr] leaves following exposure to ozone. J. Exp. Bot. 49: 1191-1202.
Booker, F. L.; Fiscus, E. L.; Miller, J. E. (1991) Ozone-induced changes in soybean cell wall physiology. In: Pell, E.; Steffen, K. , eds. Active Oxygen/Oxidative Stress and Plant Metabolism. Rockville, MD: American Society of Plant Physiologists, pp. 229-232. (Current topics in plant physiology: an American Society of Plant Physiologists series, v. 6).
Booker, F. L.; Anttonen, S.; Heagle, A. S. (1996) Catechin, proanthocyanidin and lignin contents of loblolly pint (*Pinus taeda*) needles after chronic exposure to ozone. New Phytol. 132: 483-492.
Booker, F. L.; Reid, C. D.; Brunschön-Harti, S.; Fiscus, E. L.; Miller, J. E. (1997) Photosynthesis and photorespiration in soybean [*Glycine max* (L) Merr] chronically exposed to elevated carbon dioxide and ozone. J. Exp. Bot. 48: 1843-1852.
Booker, F. L.; Burkey, K. O.; Overmyer, K.; Jones, A. M. (2004) Differential responses of G-protein *Arabidopsis thaliana* mutants to ozone. New Phytol. 162: 633-641.
Booker, F. L.; Fiscus, E. L.; Miller, J. E. (2004) Combined effects of elevated atmospheric carbon dioxide and ozone on soybean whole-plant water use. Environ. Manage. 33: S355-S362.
Booker, F. L.; Miller, J. E.; Fiscus, E. L.; Pursley, W. A.; Stefanski, L. A. (2005) Comparative responses of container- versus ground-grown soybean to elevated CO_2 and O_3. Crop Sci. 45: 883-895.
Bortier, K.; Ceulemans, R.; De Temmerman, L. (2000a) Effects of tropospheric ozone on woody plants. In: Agrawal, S. B.; Agrawal, M., eds. Environmental pollution and plant responses; pp. Lewis Publishers.; pp. 153-182.
Bortier, K.; DeTemmerman, L.; Ceulemans, R. (2000b) Effects of ozone exposure in open-top chambers on poplar (*Populus nigra*) and beech (*Fagus sylvatica*): a comparison. Environ. Pollut. 109: 509-516.
Bortier, K.; Ceulemans, R.; De Temmerman, L. (2000c) Effects of ozone exposure on growth and photosynthesis of beech seedlings (*Fagus sylvatica*). New Phytol. 146: 271-280.
Bortier, K.; Dekelver, G.; De Temmerman, L.; Ceulemans, R. (2001a) Stem injection of *Populus nigra* with EDU to study ozone effects under field conditions. Environ. Pollut. 111: 199-208.
Bortier, K.; Vandermeiren, K.; De Temmerman, L.; Ceulemans, R. (2001b) Growth, photosynthesis and ozone uptake of yound beech (*Fagus sylvatica* L) in response to different ozone exposures. Trees-Struct. Funct. 15: 75-82.
Bosac, C.; Black, V. J.; Roberts, J. A.; Black, C. R. (1998) The impact of ozone on seed yield and quality and seedling vigour in oilseed rape (*Brassica napus* L). J. Plant Physiol. 153: 127-134.
Bosley, A.; Peterson, R.; Rebbeck, J. (1998) The resistance of the moss *Polytrichum commune* to acute exposure of simulated acid rain or ozone compared to two fern species: spore germination. Bryologist 101: 512-518.
Bowler, C.; Van Montagu, M.; Inzé, D. (1992) Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol. Mol. Biol. 43: 83-116.
Bradshaw, A. D.; McNeilly, T. (1991) Evolution in relation to environmental stress. In: Taylor, G. E., Jr.; Pitelka, L. F.; Clegg, M. T., eds. Ecological genetics and air pollution. Springer-Verlag; pp. 11-31.
Braun, S.; Fluckiger, W. (1995) Effects of ambient ozone on seedlings of *Fagus sylvatica* L and *Picea abies* (L) Karst. New Phytol. 129: 33-44.
Braun, S.; Rihm, B.; Schindler, C.; Flückiger, W. (1999) Growth of mature beech in relation to ozone and nitrogen deposition: an epidemiological approach. Water Air Soil Pollut. 116: 357-364.
Brennan, E. G.; Leone, I. A.; Clarke, B. B. (1987) EDU: a chemical for evaluating ozone foliar injury and yield reduction in field-grown crops. Int. J. Trop. Plant Disease 5: 35-42.

Bringmark, E.; Bringmark, L. (1995) Disappearance of spatial variability and structure in forest floors - a distinct effect of air pollution? Water Air Soil Pollut. 85: 761-766.
Broadbent, P.; Creisson, G. P.; Kular, B.; Wellburn, A. R.; Mullineaux, P. M. (1995) Oxidative stress responses in transgenic tobacco containing altered levels of glutathione reductase activity. Plant J. 8: 247-255.
Broadmeadow, M. (1998) Ozone and forest trees. New Phytol. 139: 123-125.
Broadmeadow, M. S. J.; Heath, J.; Randle, T. J. (1999) Environmental limitations to O_3 uptake - some key results from young trees growing at elevated CO_2 concentrations. Water Air Soil Pollut. 116: 299-310.
Broadmeadow, M. S. J.; Jackson, S. B. (2000) Growth responses of *Quercus petraea*, *Fraxinus excelsior* and *Pinus sylvestris* to elevated carbon dioxide, ozone and water supply. New Phytol. 146: 437-451.
Brook, J. R.; DiGiovanni, F.; Cakmak, S.; Meyers, T. P. (1997) Estimation of dry deposition velocity using inferential models and site-specific meteorology–uncertainty due to siting of meteorological towers. Atmos. Environ. 31: 3911-3919.
Brown, V. C. (1995) Insect herbivores and gaseous air pollutants - current knowledge and predictions. In: Harrington, R.; Stork, N. E., eds. Insects in a changing environment: 17th symposium of the Royal Entomological Society. 17th symposium of the Royal Entomological Society. New York, NY: Academic Press; pp. 219-249.
Brown, K. A.; Roberts, T. M. (1988) Effects of ozone on foliar leaching in Norway spruce (*Picea abies* L Karst): confounding factors due to NO_x production during ozone generation. Environ. Pollut. 55: 55-73.
Brown, V. C.; Ashmore, M. R.; McNeill, S. (1993) Experimental investigations of the effects of air pollutants on aphids on coniferous trees. Forstwiss. Centralbl. 112: 129-132.
Brühl, C.; Crutzen, P. J. (1989) On the disproportionate role of tropospheric ozone as a filter against solar UV-B radiation. Geophys. Res. Lett. 16: 703-706.
Bruhn, J. N. (1980) Effects of oxidant air pollution on Ponderosa and Jeffrey pine foliage decomposition [dissertation]. Berkeley, CA: University of California; Dissertation Abstracts Iinternational v. 42-07B.
Brunschön-Harti, S.; Fangmeier, A.; Jäger, H. J. (1995) Influence of ozone and ethylenediurea (EDU) on growth and yield of bean (*Phaseolus vulgaris* L) in open-top field chambers. Environ. Pollut. 90: 89-94.
Buchanan, B. B.; Gruissem, W.; Jones, R. L. (2000) Biochemistry and molecular biology of plants. Rockville, MD: American Society of Plant Physiologists.
Bungener, P.; Balls, G. R.; Nussbaum, S.; Geissmann, M.; Grub, A.; Fuhrer, J. (1999a) Leaf injury characteristics of grassland species exposed to ozone in relation to soil moisture condition and vapour pressure deficit. New Phytol. 142: 271-282.
Bungener, P.; Nussbaum, S.; Grub, A.; Fuhrer, J. (1999b) Growth response of grassland species to ozone in relation to soil moisture and plant strategy. New Phytol. 142: 283-293.
Burkey, K. O. (1999) Effects of ozone on apoplast/cytoplasm partitioning of ascorbic acid in snap bean. Physiol. Plant. 107: 188-193.
Bussotti, F.; Ferretti, M. (1998) Air pollution, forest condition and forest decline in Southern Europe. Environ. Pollut. 101: 49-65.
Bytnerowicz, A. (2002) Physiological/ecological interactions between ozone and nitrogen deposition in forest ecosystems. Phyton 42: 13-28.
Bytnerowicz, A.; Turunen, M. (1994) Effects of ozone exposures on epicuticular wax of ponderosa pine needles. In: Percy, K. E.; Cape, J. N.; Jagels, R.; Simpson, C. J., eds. Air pollutants and the leaf cuticle. Springer-Verlag; pp. 305-313.
Bytnerowicz, A.; Olszyk, D. M.; Fox, C. A.; Dawson, P. J.; Kats, G.; Morrison, C. L.; Wolf, J. (1988) Responses of desert annual plants to ozone and water stress in an *in situ* experiment. JAPCA 38: 1145-1151.
Bytnerowicz, A.; Poth, M.; Takemoto, B. K. (1990) Effects of photochemical smog and mineral nutrition on ponderosa pine seedlings. Environ. Pollut. 67: 233-248.
Bytnerowicz, A.; Fenn, M. E.; Miller, P. R.; Arbaugh, M. J. (1999) Wet and dry pollutant deposition to the mixed conifer forest. In: Miller, P. R.; McBride, J. R., eds. Oxidant air pollution impacts in the montane forests of southern California: a case study of the San Bernardino Mountains. New York, NY: Springer; pp. 235-269.
Bytnerowicz, A.; Frączek, W.; Grodzińska, K.; Godzik, B.; Fleischer, P.; Krzan, Z.; Skawinski, P. (2002a) Distribution of ambient ozone concentrations in the forested areas of the Tatra Mountains. In: Widacki, W.; Bytnerowicz, A.; Riebau, A., eds. A Message from the Tatra–geographical information systems and remote sensing in mountain environmental research. Krakow, Poland: Jagiellonian University Press, 233 pp.

Bytnerowicz, A.; Godzik, B.; Frączek, W.; Grodzińska, K.; Krywult, M.; Badea, O.; Barančok, P.; Blum, O.; Černy, M.; Godzik, S.; Mankovska, B.; Manning, W.; Moravčik, P.; Musselman, R.; Oszlanyi, J.; Postelnicu, D.; Szdźuj, J.; Varšavova, M.; Zota, M. (2002b) Distribution of ozone and other air pollutants in forests of the Carpathian Mountains in central Europe. Environ. Pollut. 116: 3-25.

Bytnerowicz, A.; Tausz, M.; Alonso, R.; Jones, D.; Johnson, R.; Grulke, N. (2002c) Summer-time distribution of air pollutants in Sequoia National Park, California. Environ. Pollut. 118: 187-203.

Bytnerowicz, A.; Godzik, B.; Grodzińska, K.; Frączek, W.; Musselman, R. C.; Manning, W. J.; Badea, O.; Popescu, F.; Fleischer, P. (2004) Ambient ozone in forests of the central and eastern European mountains. Environ. Pollut. 130: 5-16.

Cairnes, M. S.; Lackey, R. T. (1992) Biodiversity and management of natural resources: the issues. Fisheries 17: 6-10.

Callaway, J. M.; Darwin, R. F.; Nesse, R. J. (1986) Economic valuation of acidic deposition damages: preliminary results from the 1985 NAPAP assessment. In: Proceedings of International Symposium on Acidic Precipitation; September; Muskoka, Ontario, Canada; Richland, WA: U. S. Environmental Protection Agency; Pacific Northwest Laboratory; Water Air Soil Pollut. 31: 1019-1034.

Campbell, S.; Temple, P.; Pronos, J.; Rochefort, R.; Andersen, C. (2000) Monitoring for ozone injury in west coast (Oregon, Washington, California) forests in 1998. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station; general technical report no. PNW-GTR-495. Available: http://www.fs.fed.us/pnw/gtrs.htm [11 April, 2003].

Cao, W.; Tibbitts, T. W.; Wheeler, R. M. (1994) Carbon dioxide interactions with irradiance and temperature in potatoes. In: MacElroy, R. D. Life sciences and space research XXV(3): natural and artificial ecosystems: proceedings of the topical meeting of the COSPAR Interdisciplinary Scientific Commission F (Meeting F4) of the COSPAR twenty-ninth plenary meeting; August-September, 1992; Washington, DC. New York, NY: Committee on Space Research; pp. 243-250. (Advances in space research: v. 14, no. 11).

Cape, J. N. (1996) Surface wetness and pollutant deposition. In: Kerstiens, G., ed. Plant cuticles: an integrated functional approach. an integrated functional approach. Oxford, United Kingdom: BIOS Scientific Publishers; pp. 283-300.

Cardoso-Vilhena, J.; Balaguer, L.; Daymond, J.; Barnes, J. D. (1998) Amelioration of ozone damage by elevated CO_2. In: DeKok, L. J.; Stulen, I., eds. Responses of plant metabolism to air pollution and global change. Leiden, The Netherlands: Backhuys Publishers; pp. 281-282.

Cardoso-Vilhena, J.; Barnes, J. (2001) Does nitrogen supply affect the response of wheat (*Triticum aestivum* cv Hanno) to the combination of elevated CO_2 and O_3? J. Exp. Bot. 52: 1901-1911.

Carey, W. A.; Kelley, W. D. (1994) Interaction of ozone exposure and *Fusarium subglutinans* inocculation on growth and disease development of loblolly pine seedlings. Environ. Pollut. 84: 35-43.

Carnahan, J. E.; Jenner, E. L.; Wat, E. K. W. (1978) Prevention of ozone injury to plants by a new protectant chemical. Phytopathology 68: 1225-1229.

Carpenter, R. A.; Dixon, J. A. (1985) Ecology meets economics: a guide to sustainable development. Environment 27: 6-32.

Carroll, J. J.; Miller, P. R.; Pronos, J. (2003) Historical perspectives on ambient ozone and its effects on the Sierra Nevada. In: Bytnerowicz, A.; Arbaugh, M. J.; Alonso, R., eds. Ozone air pollution in the Sierra Nevada: distribution and effects on forests; pp. distribution and effects on forests; pp. 33-54.

Castillo, F. J.; Greppin, H. (1986) Balance between anionic and cationic extracellular peroxidase activities in *sedum album* leaves after ozone exposure Analysis by high-performance liquid chromatography. Physiol. Plant. 68: 201-208.

Castillo, F. J.; Heath, R. L. (1990) CA^{2+} transport in membrane vesicles from pinto bean leaves and its alteration after ozone exposure. Plant Physiol. 94: 788-795.

Castillo, F. J.; Penel, C.; Greppin, H. (1984) Peroxidase release induced by ozone in *sedum album* leaves: involvement of Ca2+. Plant Physiol. 74: 846-851.

Castillo, F. J.; Miller, P. R.; Greppin, H. (1987) Extracellular biochemical markers of photochemical oxidant air pollution damage to Norway spruce. Experientia (Basel) 43: 111-115.

Castle, E. N. (1993) A pluralistic, pragmatic and evolutionary approach to natural resource management. For. Ecol. Manage. 56: 279-295.

Cazalé, A. C.; Rouet-Mayer, M. A.; Barbier-Brygoo, H.; Mathieu, Y.; Laurière, C. (1998) Oxidatiave burst and hypoosmotic stress in tobacco cell suspensions. Plant Physiol. 116: 659-669.

Chappelka, A. H. (2002) Reproductive development of blackberry (*Rubus cuneifolius*) as influenced by ozone. New Phytol. 155: 249-255.

Chappelka, A. H., III; Chevone, B. I. (1986) White ash seedling growth response to ozone and simulated acid rain. Can. J. For. Res. 16: 786-790.
Chappelka, A. H.; Freer-Smith, P. H. (1995) Predisposition of trees by air pollutants to low temperatures and moisture stress. Environ. Pollut. 87: 105-117.
Chappelka, A. H.; Samuelson, L. J. (1998) Ambient ozone effects on forest trees of the eastern United States: a review. New Phytol. 139: 91-108.
Chappelka, A. H., III; Chevone, B. I.; Burk, T. E. (1985) Growth response of yellow-poplar (*Liriodendron tulipifera* L) seedlings to ozone, sulfur dioxide, and simulated acidic precipitation, alone and in combination. Environ. Exp. Bot. 25: 233-244.
Chappelka, A.; Renfro, J.; Somers, G.; Nash, B. (1997) Evaluation of ozone injury on foliage of black cherry (*Prunus serotina*) and tall milkweed (*Asclepias exaltata*) in Great Smoky Mountains National Park. Environ. Pollut. 95: 13-18.
Chappelka, A.; Skelly, J.; Somers, G.; Renfro, J.; Hildebrand, E. (1999a) Mature black cherry used as a bioindicator of ozone injury. Water Air Soil Pollut. 116: 261-266.
Chappelka, A.; Somers, G.; Renfro, J. (1999b) Visible ozone injury on forest trees in Great Smoky Mountains National Park, USA. Water Air Soil Pollut. 116: 255-260.
Chappelka, A. H.; Neufeld, H. S.; Davison, A. W.; Somers, G. L.; Renfro, J. R. (2003) Ozone injury on cutleaf coneflower (*Rudbeckia laciniata*) and crown-beard (*Verbesina occidentalis*) in Great Smoky Mountains National Park. Environ. Pollut. 125: 53-60.
Chen, C. W.; Tsai, W. T.; Gomez, L. E. (1994) Modeling responses of Ponderosa pine to interacting stresses of ozone and drought. For. Sci. 40: 267-288.
Chen, C. W.; Tsai, W. T.; Lucier, A. A. (1998) A model of air-tree-soil system for ozone impact analysis. Ecol. Modell. 111: 207-222.
Cheong, Y. H.; Chang, H. S.; Gupta, R.; Wang, X.; Zhu, T.; Luan, S. (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in arabidopsis. Plant Physiol. 129: 661-677.
Chernikova, T.; Robinson, J. M.; Lee, E. H.; Mulchi, C. L. (2000) Ozone tolerance and antioxidant enzyme activity in soybean cultivars. Photosynth. Res. 64: 15-26.
Chevrier, N.; Chung, Y. S.; Sarhan, F. (1990) Oxidative damages and repair in *Euglena gracilis* exposed to ozone: II membrane permeability and uptake of metabolites. Plant Cell Physiol. 31: 987-992.
Clark, C. S.; Weber, J. A.; Lee, E. H.; Hogsett, W. E. (1995) Accentuation of gas exchange gradients in flushes of ponderosa pine exposed to ozone. Tree Physiol. 15: 181-189.
Clarke, B. B.; Henninger, M. R.; Brennan, E. (1983) An assessment of potato losses caused by oxidant air pollution in New Jersey. Phytopathology 73: 104-108.
Cobb, F. W., Jr.; Wood, D. L.; Stark, R. W.; Parmeter, J. R., Jr. (1968) Photochemical oxidant injury and bark beetle (*Coleoptera: Scolytidae*) infestation of ponderosa pine IV Theory on the relationships between oxidant injury and bark beetle infestation. Hilgardia 39: 141-152.
Coe, H.; Gallagher, M. W.; Choularton, T. W.; Dore, C. (1995) Canopy scale measurements of stomatal and cuticular O_3 uptake by Sitka spruce. Atmos. Environ. 29: 1413-1423.
Coleman, J. S.; Jones, C. G.; Smith, W. H. (1987) The effect of ozone on cottonwood - leaf rust interactions: independence of abiotic stress, genotype, and leaf ontogeny. Can. J. Bot. 65: 949-953.
Coleman, M. D.; Dickson, R. E.; Isebrands, J. G.; Karnosky, D. F. (1995a) Carbon allocation and partitioning in aspen clones varying in sensitivity to tropospheric ozone. Tree Physiol. 15: 593-604.
Coleman, M. D.; Isebrands, J. G.; Dickson, R. E.; Karnosky, D. F. (1995b) Photosynthetic productivity of aspen clones varying in sensitivity to tropospheric ozone. Tree Physiol. 15: 585-592.
Coleman, M. D.; Dickson, R. E.; Isebrands, J. G.; Karnosky, D. F. (1996) Root growth physiology of potted and field-grown trembling aspen exposed to tropospheric ozone. Tree Physiol. 16: 145-152.
Colls, J. J.; Unsworth, M. H. (1992) Air pollution interactions with natural stressors. In: Barker, J. R.; Tingey, D. T., eds. Air pollution effects on biodiversity. New York, NY: Van Nostrand Reinhold; pp. 93-108.
Common, M.; Perrings, C. (1992) Towards an ecological economics of sustainability. Ecol. Econ. 6: 7-34.
Conklin, P. L.; Barth, C. (2004) Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. Plant Cell Environ. 27: 959-970.
Conklin, P. L.; Last, R. L. (1995) Differential accumulation of antioxidant mRNAs in *Arabidopsis thaliana* exposed to ozone. Plant Physiol. 109: 203-212.
Conklin, P. L.; Williams, E. H.; Last, R. L. (1996) Environmental stress sensitivity of an ascorbic acid-deficient Arabidopsis mutant. Proc. Natl. Acad. Sci. U. S. A. 93: 9970-9974.

Constable, J. V. H.; Friend, A. L. (2000) Suitability of process-based tree growth models for addressing tree response to climate change. Environ. Pollut. 110: 47-59.
Constable, J. V. H.; Retzlaff, W. A. (1997) Simulating the response of mature yellow poplar and loblolly pine trees to shifts in peak ozone periods during the growing season using the TREGRO model. Tree Physiol. 17: 627-635.
Constable, J. V. H.; Taylor, G. E., Jr. (1997) Modeling the effects of elevated tropospheric O_3 on two varieties of *Pinus ponderosa*. Can. J. For. Res. 27: 527-537.
Constable, J. V. H.; Taylor, G. E., Jr.; Laurence, J. A.; Weber, J. A. (1996) Climatic change effects on the physiology and growth of *Pinus ponderosa*: expectations from simulation modeling. Can. J. For. Res. 26: 1315-1325.
Cook, E. (1990) A conceptual linear aggregate model for tree rings. In: Cook, E.; Kairiukstis, A., eds. Methods of dendrochronology: applications in the environmental sciences. Dordrecht, The Netherlands: Kluwer Academic Publ.; pp. 98-104.
Cooley, D. R.; Manning, W. J. (1987) The impact of ozone on assimilate partitioning in plants: a review. Environ. Pollut. 47: 95-113.
Coruzzi, G. M.; Zhou, L. (2001) Carbon and nitrogen sensing and signaling in plants: emerging 'matrix effects'. Curr. Opin. Plant Biol. 4: 247-253.
Costa, S. D.; Kennedy, G. G.; Heagle, A. S. (2001) Effect of host plant ozone stress on Colorado potato beetles. Environ. Entomol. 30: 824-831.
Costanza, R.; d'Arge, R.; De Groot, R.; Farber, S.; Grasso, M.; Hannon, B.; Limburg, K.; Naeem, S.; O'Neill, R. V.; Paruelo, J.; Raskin, R. G.; Sutton, P.; Van Den Belt, M. (1997) The value of the world's ecosystem services and natural capital. Nature (London) 387: 253-259.
Coulston, J. W.; Smith, G. C.; Smith, W. D. (2003) Regional assessment of ozone sensitive tree species using bioindicator plants. Environ. Monit. Assess. 83: 113-127.
Cox, R. M.; Malcolm, J. W. (1999) Passive ozone monitoring for forest health assessment. Water Air Soil Pollut. 116: 339-344.
Craigon, J.; Fangmeier, A.; Jones, M.; Donnelly, A.; Bindi, M.; De Temmerman, L.; Persson, K.; Ojanpera, K. (2002) Growth and marketable-yield responses of potato to increased CO_2 and ozone. Eur. J. Agron. 17: 273-289.
Crocker, T. D. (1985) On the value of the condition of a forest stock. Land Econ. 61: 244-254.
Crocker, T. D.; Forster, B. A. (1986) Atmospheric deposition and forest decline. Water Air Soil Pollut. 31: 1007-1017.
Croft, P. C.; Jüttner, F.; Slusarenko, A. J. (1993) Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L) leaves inoculated with *pseudomonas syringae* pv *phaseolicola*. Plant Physiol. 101: 13-24.
Curtis, P. S. (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. Plant Cell Environ. 19: 127-137.
Dahlsten, D. L.; Rowney, D. L.; Kickert, R. N. (1997) Effects of oxidant air pollutants on western pine beetle (Coleoptera: Scolytidae) populations in southern California. Environ. Pollut. 96: 415-423.
Daily, G. C. (1997) Nature's services: societal dependence on natural ecosystems.
Daily, G. C. (1999) Developing a scientific basis for managing Earth's life support systems. Conserv. Ecol. 3: 14.
Daily, G. C. (2000) Ecological assessment then and now. Sci. Technol. 16: 6-15.
Dämmgen, U.; Grünhage, L.; Haenel, H. D.; Jäger, H. J. (1993) Climate and stress in ecotoxicology: a coherent system of definitions and terms. Angew Bot. 67: 157-162.
Dämmgen, U.; Grünhage, L.; Kusters, A.; Jäger, H. J. (1994) Response of a grassland ecosystem to air pollutants:--II the chemical climate: fluxes of sedimenting airborne matter. Environ. Pollut. 85: 35-42.
Danielsson, H.; Gelang, J.; Pleijel, H. (1999) Ozone sensitivity, growth and flower development in *Phleum* genotypes of different geographic origin in the Nordic countries. Environ. Exp. Bot. 42: 41-49.
Danielsson, H.; Karlsson, G. P.; Karlsson, P. E.; Pleijel, J. (2003) Ozone uptake modelling and flux-response relationships–an assessment of ozone-induced yield loss in spring wheat. Atmos. Environ. 37: 475-485.
Darrall, N. M. (1989) The effect of air pollutants on physiological processes in plants. Plant Cell Environ. 12: 1-30.
Dass, H. C.; Weaver, G. M. (1968) Modification of ozone damage to *Phaseolus vulgaris* by antioxidants, thiols and sulfhydryl reagents. Can. J. Plant Sci. 48: 569-574.
Dass, H. C.; Weaver, G. M. (1972) Enzymatic changes in intact leaves of *Phaseolus vulgaris* following ozone fumigation. Atmos. Environ. 6: 759-763.
Davidson, A. (1993) Update of ozone trends in California's South Coast Air Basin. Air Waste 43: 226-227.

Davis, D. D.; Skelly, J. M. (1992) Foliar sensitivity of eight eastern hardwood tree species to ozone. Water Air Soil Pollut. 62: 269-277.
Davison, A. W.; Barnes, J. D. (1998) Effects of ozone on wild plants. New Phytol. 139: 135-151.
Davison, A. W.; Reiling, K. (1995) A rapid change in ozone resistance of *Plantago major* after summers with high ozone concentrations. New Phytol. 131: 337-344.
Davison, A. W.; Neufeld, H. S.; Chappelka, A. H.; Wolff, K. W.; Finkelstein, P. L. (2003) Interpreting spatial variation in ozone symptoms shown by cutleaf cone flower, *Rudbeckia laciniata* L. Environ. Pollut. 125: 61-70.
De Bauer, L. I. (1972) Uso de plantas indicadoras en la determinacion de aeropolutos en la Ciudad de México [Use of indicative plants to determine air pollution in Mexico city]. Agrociencia 9: 139-141.
De Bauer, L. I.; Hernández-Tejeda, T. (1986) Contaminación: una amenaza para la vegetación en México. Chapingo, Mexico: Colegio de Postgraduados.
De Bauer, L. I.; Krupa, S. V. (1990) The Valley of Mexico: summary of observational studies of air quality and effects on vegetation. Environ. Pollut. 65: 109-118.
De Kok, L. J.; Stulen, I. (1998) Responses of plant metabolism to air pollution and global change. Leiden, The Netherlands: Backhuys Publishers.
De Kok, L. J.; Tausz, M. (2001) The role of glutathione in plant reaction and adaptation to air pollutants. In: Grill, D.; Tausz, M.; De Kok, L. J., eds. Significance of glutathione in plant adaptation to the environment. Amsterdam, The Netherlands: Kluwer Publishers; pp. 185-208.
De Ruiter, P. C.; Neutel, A. M.; Moore, J. C. (1998) Biodiversity in soil ecosystems: the role of energy flow and community stability. Appl. Soil Ecol. 10: 217-228.
De Santis, F. (1999) New directions: will a new European vegetation ozone standard be fair to all European countries? Atmos. Environ. 33: 3873-3874.
De Santis, F. (2000) Reply to 'Use and abuse of the AOT40 concept' by M Ashmore and J Fuhrer [letter]. Atmos. Environ. 34: 1158-1159.
De Temmerman, L.; Hacour, A.; Guns, M. (2002a) Changing climate and potential impacts on potato yield and quality 'CHIP': introduction, aims and methodology. Eur. J. Agron. 17: 233-242.
De Temmerman, L.; Wolf, J.; Colls, J.; Bindi, M.; Fangmeier, A.; Finnan, J.; Ojanperä, K.; Pleijel, H. (2002b) Effect of climatic conditions on tuber yield (*Solanum tuberosum* L) in the European 'CHIP' experiments. Eur. J. Agron. 17: 243-255.
DeCaria, A. J.; Pickering, K. E.; Stenchikov, G. L.; Scala, J. R.; Stith, J. L.; Dye, J. E.; Ridley, B. A.; Laroche, P. (2000) A cloud-scale model study of lightning-generate NO_x in an individual thunderstorm during STERAO-A. J. Geophys. Res. [Atmos.] 105: 11601-11616.
Degousée, N.; Triantaphylidès, C.; Montillet, J. L. (1994) Involvement of oxidative processes in the signaling mechanisms leading to the activation of glyceollin synthesis in soybean (*Glycine max*). Plant Physiol. 104: 945-952.
DeLucia, E. H.; Hamilton, J. G.; Naidu, S. L.; Thomas, R. B.; Andrews, J. A.; Finzi, A.; Lavine, M.; Matamala, R.; Mohan, J. E.; Hendrey, G. R.; Schlesinger, W. H. (1999) Net primary production of a forest ecosystem with experimental CO_2 enrichment. Science (Washington, DC) 284: 1177-1179.
Desikan, R.; Mackerness, S.; Hancock, J. T.; Neill, S. J. (2001) Regulation of the *Arabidopsis transcriptome* by oxidative stress. Plant Physiol. 127: 159-172.
Diaz, G.; Barrantes, O.; Honrubia, M.; Gracia, C. (1996) Effect of ozone and sulphur dioxide on mycorrhizae of *Pinus halepensis* Miller. Ann. Sci. For. 53: 849-856.
Dickson, R. E.; Isebrands, J. G. (1991) Leaves as regulators of stress response. In: Mooney, H. A.; Winner, W. E.; Pell, E. J., eds. Response of plants to multiple stresses. Academic Press, Inc.; pp. 3-34.
Dickson, R. E.; Coleman, M. D.; Reimenschneider, D. E.; Isebrands, J. G.; Hogan, G. D.; Karnosky, D. F. (1998) Growth of five hybrid poplar genotypes exposed to interacting elevated CO_2 and O_3. Can. J. For. Res. 28: 1706-1716.
Dickson, R. E.; Coleman, M. D.; Pechter, P.; Karnosky, D. (2001) Growth and crown architecture of two aspen genotypes exposed to interacting ozone and carbon dioxide. Environ. Pollut. 115: 319-334.
Dietz, K. J. (1997) Functions and responses of the leaf apoplast under stress. Prog. Bot. 58: 221-254.
Dighton, J.; Jansen, A. E. (1991) Atmospheric pollutants and ectomycorrhizae: more questions than answers? Environ. Pollut. 73: 179-204.
Dijak, M.; Ormrod, D. P. (1982) Some physiological and anatomical characteristics associated with differential ozone sensitivity among pea cultivars. Environ. Exp. Bot. 22: 395-402.

Dixon, B. L.; Segerson, K. (1999) Impacts of increased climate variability on the profitability of Midwest agriculture. J. Agric. Appl. Econ. 31: 537-550.
Dixon, M.; Le Thiec, D.; Garrec, J. P. (1998) Reactions of Norway spruce and beech trees to 2 years of ozone exposure and episodic drought. Environ. Exp. Bot. 40: 77-91.
Docherty, M.; Salt, D. T.; Holopainen, J. K. (1997) The impacts of climate change and pollution on forest pests. In: Watt, A. D.; Stork, N. E.; Hunter, M. D., eds. Forests and insects. Chapman and Hall; pp. 229-247.
Dominy, P. J.; Heath, R. L. (1985) Inhibition of the K^+-stimulated ATpase of the plasmalemma of pinto bean leaves by ozone. Plant Physiol. 77: 43-45.
Donnelly, A.; Jones, M. B.; Schneiders, B. J.; Burke, J. I. (1998) The interactive effects of CO_2, O_3 and nitrogen on the photosynthetic response of juvenile wheat plants. In: De Kok, L. J.; Stulen, I., eds. Responses of plant metabolism to air pollution and global change. Backhuys Publishers; pp. 291-293.
Donnelly, A.; Jones, M. B.; Burke, J. I.; Schnieders, B. (1999) Does elevated CO_2 protect grain yield of wheat from the effects of ozone stress? Z. Naturforsch. C: J. Biosci. 54: 802-811.: J-811.
Donnelly, A.; Jones, M. B.; Burke, J. I.; Schneiders, B. (2000) Elevated CO_2 provides protection from O_3 induced photosynthetic damage and chlorophyll loss in flag leaves of spring wheat (*Triticum aestivum* L, cv'Minaret'). Agric. Ecosyst. Environ. 80: 159-168.
Donnelly, A.; Craigon, J.; Black, C. R.; Colls, J. J.; Landon, G. (2001a) Does elevated CO_2 ameliorate the impact of O_3 on the chlorophyll content and photosynthesis in potato (*Solanum tuberosum*)? Physiol. Plant. 111: 501-511.
Donnelly, A.; Craigon, J.; Black, C. R.; Colls, J. J.; Landon, G. (2001b) Elevated CO_2 increases biomass and tuber yield in potato even at high ozone concentrations. New Phytol. 149: 265-274.
Donnelly, A.; Lawson, T.; Craigon, J.; Black, C. R.; Coils, J. J.; Landon, G. (2001b) Effects of elevated CO_2 and O_3 on tuber quality in potato (*Solanum tuberosum* L). Agric. Ecosyst. Environ. 87: 273-285.
Dowding, P. (1988) Air pollutant effects on plant pathogens. In: Schulte-Hostede, S.; Darrall, N. M.; Blank, L. W.; Wellburn, A. R., eds. Air pollution and plant metabolism. London, United Kingdom: Elsevier Applied Scientific; pp. 329-355.
Drogoudi, P. D.; Ashmore, M. R. (2000) Does elevated ozone have differing effects in flowering and deblossomed strawberry? New Phytol. 147: 561-569.
Drogoudi, P. D.; Ashmore, M. (2001) ^{14}C-allocation of flowering and deblossomed strawberry in response to elevated ozone. New Phytol. 152: 455-461.
Duchelle, S. F.; Skelly, J. M. (1981) Response of common milkweed to oxidant air pollution in the Shenandoah National Park in Virginia. Plant Dis. 65: 661-663.
Duchelle, S. F.; Skelly, J. M.; Chevone, B. I. (1982) Oxidant effects on forest tree seedling growth in the Appalachian mountains. Water Air Soil Pollut. 18: 363-373.
Duchelle, S. F.; Skelly, J. M.; Sharick, T. L.; Chevone, B. I.; Yang, Y. S.; Nellessen, J. E. (1983) Effects of ozone on the productivity of natural vegetation in a high meadow of the Shenandoah National Park of Virginia. J. Environ. Manage. 17: 299-308.
Dueck, T.; Zuin, A.; Elderson, J. (1998) Influence of ammonia and ozone on growth and drought sensitivity of *Pinus sylvestris*. Atmos. Environ. 32: 545-550.
Dugger, W. M.; Ting, I. P. (1970) Air pollution oxidants–their effects on metabolic processes in plants. Ann. Rev. Plant Physiol. 21: 215-234.
Dunajski, A. (2002) Mountain forest vegetation dynamics after huge forest dieback: landscape level approach in the Polish part of Karkonosze Mountains. In: Widacki, W.; Bytnerowicz, A.; Riebau, A., eds. A message from the Tatra–geographical information systems and remote sensing in mountain environmental research. Krakow, Poland: Jagiellonian University Press, 233 pp.
Dunn, D. B. (1959) Some effects of air pollution on *Lupinus* in the Los Angeles area. Ecology 40: 621-625.
Eamus, D. (1996) Responses of field grown trees to CO_2 enrichment. Commonw. For. Rev. 75: 39-47.
Eamus, D.; Murray, M. (1991) Photosynthetic and stomatal conductance responses of Norway spruce and beech to ozone, acid mist and frost - a conceptual model. Environ. Pollut. 72: 23-44.
Eckey-Kaltenbach, H.; Ernst, D.; Heller, W.; Sandermann, H., Jr. (1994a) Biochemical responses to ozone IV Cross-induction of defensive pathways in parsley (*Petroselinum crispum* L) plants. Plant Physiol. 104: 67-74.
Eckey-Kaltenbach, H.; Grosskopf, E.; Sandermann, H., Jr.; Ernst, D. (1994b) Induction of pathogen defence genes in parsley (*Petroselinum crispum* L) plants by ozone. Proc. R. Soc. Edinburgh Sect. B: Biol. Sci. 102: 63-74.: Biol-74.

Edinger, J. G.; McCutchan, M. H.; Miller, P. R.; Ryan, B. C.; Schroeder, M. J.; Behar, J. V. (1972) Penetration and duration of oxidant air pollution in the South Coast Air Basin of California. J. Air Pollut. Control Assoc. 22: 882-886.

Edwards, N. T. (1991) Root and soil respiration responses to ozone in *Pinus taeda* L seedlings. New Phytol. 118: 315-321.

Edwards, G. S.; Kelly, J. M. (1992) Ectomycorrhizal colonization of loblolly pine seedlings during three growing seasons in response to ozone, acidic precipitation, and soil Mg status. Environ. Pollut. 76: 71-77.

Edwards, G. S.; Wullschleger, S. D.; Kelly, J. M. (1994) Growth and physiology of northern red oak: preliminary comparisons of mature tree and seedling responses to ozone. Environ. Pollut. 83: 215-221.

Einig, W.; Lauxmann, U.; Hauch, B.; Hampp, R.; Landolt, W.; Maurer, S.; Matyssek, R. (1997) Ozone-induced accumulation of carbohydrates changes enzyme activities of carbohydrate metabolism in birch leaves. New Phytol. 137: 673-680.

Elagöz, V.; Manning, W. J. (2002) Bean plants and ozone: morphology matters. Environ. Pollut. 120: 521-524.

Elagöz, V.; Manning, W. J. (2005a) Factors affecting the effects of EDU on growth and yield of field-grown bush beans (*Phaseolus vulgaris* L), with varying degrees of sensitivity to ozone. Environ. Pollut. 136: 385-395.

Elagöz, V.; Manning, W. J. (2005b) Responses of sensitive and tolerant bush beans (*Phaseolus vulgaris* L.) to ozone in open-top chambers are influenced by phenotypic differences, morphological characteristics, and the chamber environment. Environ. Pollut. 136: 371-383.

Elkiey, T.; Ormrod, D. P. (1979) Leaf diffusion resistance responses of three petunia cultivars to ozone and/or sulfur dioxide. J. Air Pollut. Control Assoc. 29: 622-625.

Elkiey, T.; Ormrod, D. P. (1981) Sorption of O_3, SO_2, NO_2 or their mixture by nine *Poa pratensis* cultivars of differing pollutant sensitivity. Atmos. Environ. 15: 1739-1743.

Ellsworth, D. S.; Reich, P. B.; Naumburg, E. S.; Koch, G. W.; Kubiske, M. E.; Smith, S. D. (2004) Photosynthesis, carboxylation and leaf nitrogen response of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. Global Change Biol. 10: 2121-2138.. Global Change Biol. 10: 2121-2138.

Elvira, S.; Bermejo, V.; Manrique, E.; Gimeno, B. S. (2004) On the response of two populations of *Quercus coccifera* to ozone and its relationship with ozone uptake. Atmos. Environ. 38: 2305-2311.

Emberson, L.; Ashmore, M. R.; Cambridge, H. M.; Simpson, D.; Tuovinen, J. P. (2000a) Modelling stomatal ozone flux across Europe. Environ. Pollut. 109: 403-413.

Emberson, L. D.; Wieser, G.; Ashmore, M. R. (2000b) Modelling of stomatal conductance and ozone flux of Norway spruce: comparison with field data. Environ. Pollut. 109: 393-402.

Enders, G. (1992) Deposition of ozone to a mature spruce forest: measurements and comparison to models. Environ. Pollut. 75: 61-67.

Engle, R. L.; Gabelman, W. H. (1966) Inheritance and mechanism for resistance to ozone damage in onion, *Allium cepa* L. Proc. Am. Soc. Hortic. Sci. 89: 423-430.

Ennis, C. A.; Lazrus, A. L.; Kok, G. L.; Zimmerman, P. R.; Monson, R. K. (1990) A branch chamber system and techniques for simultaneous pollutant exposure experiments and gaseous flux determinations. Tellus Ser. B 42: 170-182.

Ernst, D.; Schraudner, M.; Langebartels, C.; Sandermann, H. (1992) Ozone-induced changes of messenger RNA levels of beta-1,3-glucanase, chitinase and pathogenesis-related protein 1B in tobacco plants. Plant. Mol. Biol. 20: 673-682.

Espelie, K. E.; Franceschi, V. R.; Kolattukudy, P. E. (1986) Immunocytochemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound-healing potato tuber tissue. Plant Physiol. 81: 487-492.

Estes, B. L.; Enebak, S. A.; Chappalka, A. H. (2004) Loblolly pine seedling growth after inoculation with plant growth-promoting rhizobacteria and ozone exposure. Can. J. For. Res. 34: 1410-1416.

Evans, P. A.; Ashmore, M. R. (1992) The effects of ambient air on a semi-natural grassland community. Agric. Ecosyst. Environ. 38: 91-97.

Ewart, F.; Pleijel, H. (1999) Phenological development, leaf emergence, tillering and leaf area index, and duration of spring wheat across Europe in response to CO_2 and ozone. Eur. J. Agron. 10: 171-184.

Ewart, F.; Van Oijen, M.; Porter, J. R. (1999) Simulation of growth and development processes of spring wheat in response to CO_2 and ozone for different sites and years in Europe using mechanistic crop simulation models. Eur. J. Agron. 10: 231-247.

Fagnano, M.; Merola, G.; Forlani, A.; Postiglione, L.; Fuhrer, J. (2004) Monitoring yield loss from ozone pollution in a Mediterranean environment: a comparison of methods. Water Air Soil Pollut. 155: 383-398.

Fangmeier, A.; Stein, W.; Jäger, H.-J. (1992) Advantages of an open-top chamber plant exposure system to assess the impact of atmospheric trace gases on vegetation. Angew Bot. 66: 97-105.
Fangmeier, A.; Brockerhoff, U.; Grüters, U.; Jäger, H. J. (1994a) Growth and yield responses of spring wheat (*Triticum aestivum* L cv Turbo) grown in open-top chambers to ozone and water stress. Environ. Pollut. 83: 317-325.
Fangmeier, A.; Brunschön, S.; Jäger, H. J. (1994b) Time course of oxidant stress biomarkers in flag leaves of wheat exposed to ozone and drought stress. New Phytol. 126: 63-69.
Fangmeier, A.; Grüters, U.; Vermehren, B.; Jäger, H. J. (1996) Responses of some cereal cultivars to CO_2 enrichment and tropospheric ozone at different levels of nitrogen supply. J. Appl. Bot. 70: 12-18.
Fangmeier, A.; De Temmerman, L.; Mortensen, L.; Kemp, K.; Burke, J.; Mitchell, R.; Van Oijen, M.; Weigel, H. J. (1999) Effects on nutrients and on grain quality in spring wheat crops grown under elevated CO_2 concentrations and stress conditions in the European, multiple-site experiment 'ESPACE-wheat'. Eur. J. Agron. 10: 215-229.
Fangmeier, A.; De Temmerman, L.; Black, C.; Persson, K.; Vorne, V. (2002) Effects of elevated CO_2 and/or ozone on nutrient concentrations and nutrient uptake of potatoes. Eur. J. Agron. 17: 353-368.
Fangmeier, A.; Bender, J.; Weigel, H. J.; Jäger, H. J. (2002) Effects of pollutant mixtures. In: Bell, J. N. B.; Treshow, M., eds. Air Pollution and Plant Life. 2nd ed. Chichester, United Kingdom: John Wiley & Sons Ltd.; pp. 251-272.
Farage, P. K.; Long, S. P. (1999) The effects of O_3 fumigation during leaf development on photosynthesis of wheat and pea: an *in vivo* analysis. Photosynth. Res. 59: 1-7.
Farage, P. K.; Long, S. P.; Lechner, E. G.; Baker, N. R. (1991) The sequence of changes within the photosynthetic apparatus of wheat following short-term exposure to ozone. Plant Physiol. 95: 529-535.
Farquhar, G. D.; Sharkey, T. D. (1982) Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. 33: 317-345.
Farquhar, G. D.; Caemmerer, S. V.; Berry, J. A. (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149: 78-90.
Federal Land Manager's Air Quality Related Values Workgroup (FLAG) (2000) Phase I report. Denver, CO: National Park Service, Air Resources Division; Available: http://www2.nature.nps.gov/ard/flagfree/ [11 March, 2003].
Federal Register. (1997) National ambient air quality standards for ozone; final rule. F.R. 62: 38855-38896.
Fenn, M. E.; De Bauer, L. I. (1999) Nitogen and sulfur deposition and forest nutrient status in the Valley of Mexico. Water Air Soil Pollut. 113: 155-174.
Fenn, M. E.; Dunn, P. H. (1989) Litter decomposition across an air-pollution gradient in the San Bernardino Mountains. Soil Sci. Soc. Am. J. 53: 1560-1567.
Fenn, M. E.; Poth, M. A. (1998) Indicators of nitrogen status in California forests. U. S. D. A. Forest Service Gen. Tech. Rep. PSW-GTR-166.
Fenn, M. E.; Dunn, R. H.; Wilborn, R. (1990) Black stain root disease in ozone-stressed ponderosa pine. Plant Dis. 74: 426-430.
Fenn, M. E.; Poth, M. A.; Johnson, D. W. (1996) Evidence for nitrogen saturation in the San Bernardino Mountains in southern California. For. Ecol. Manage. 82: 211-230.
Fenn, M. E.; Poth, M. A.; Aber, J. D.; Baron, J. S.; Bormann, B. T.; Johnson, D. W.; Lemly, A. D.; McNulty, S. G.; Ryan, D. F.; Stottlemyer, R. (1998) Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. Ecol. Appl. 8: 706-733.
Fenn, M. E.; Poth, M. A.; Schilling, S. L.; Grainger, D. B. (2000) Throughfall and fog deposition of nitrogen and sulfur at an N-limited and N-saturated site in the San Bernardino Mountains, southern California. Can. J. For. Res. 30: 1476-1488.
Fenn, M. E.; De Bauer, L. I.; Hernández-Tejeda, T. (2002) Urban air pollution and forests: resources at risk in the Mexico City air basin. New York, NY: Springer-Verlag (Ecological Studies Vol. 156).
Fenn, M. A.; Haeuber, R.; Tonnesen, G. S.; Baron, J. S.; Grossman-Clarke, S.; Hope, D.; Jaffe, D. A.; Copeland, S.; Geiser, L.; Rueth, H. M.; Sickman, J. O. (2003a) Nitrogen emissions, deposition, and monitoring in the western United States. BioScience 53: 391-403.
Fenn, M. E.; Baron, J. S.; Allen, E. B.; Rueth, H. M.; Nydick, K. R.; Geiser, L.; Bowman, W. D.; Sickman, J. O.; Meixner, T.; Johnson, D. W.; Neitlich, P. (2003b) Ecological effects of nitrogen deposition in the western United States. BioScience 53: 404-420.
Ferdinand, J. A.; Fredericksen, T. S.; Kouteric, K. B.; Skelly, J. M. (2000) Leaf morphology and ozone sensitivity of two open pollinated genotypes of black cherry (*Prunus serotina*) seedlings. Environ. Pollut. 108: 297-302.

Findlay, S.; Jones, C. G. (1990) Exposure of cottonwood plants to ozone alters subsequent decomposition. Oecologia 82: 248-250.
Findlay, S.; Carreiro, M.; Jones, C. G. (1991) Ozone stress and mite damage to cottonwood plants alter subsequent litter decomposition: the afterlife effect. Bull. Ecol. Soc. Am. 72(suppl.): 113.
Findlay, S.; Carreiro, M.; Krischik, V.; Jones, C. G. (1996) Effects of damage to living plants on leaf litter quality. Ecol. Appl. 6: 269-275.
Findley, D. A.; Keever, G. J.; Chappelka, A. H.; Eakes, D. J.; Gillian, D. J. (1997) Differential responses of buddleia (*Buddleia davidii* Franch.) to ozone. Environ. Pollut. 98: 105-111.
Finkelstein, R. R.; Gibson, S. I. (2002) ABA and sugar interactions regulating development: cross-talk or voices in a crowd? Curr. Opin. Plant Biol. 5: 26-32.
Finnan, J. M.; Burke, J. L.; Jones, M. B. (1997) An evaluation of indices that describe the impact of ozone on the yield of spring wheat (*Triticum aestivum* L). Atmos. Environ. 31: 2685-2693.
Finnan, J. M.; Donnelly, A.; Burke, J. I.; Jones, M. B. (2002) The effects of elevated concentrations of carbon dioxide and ozone on potato (*Solanum tuberosum* L) yield. Agric. Ecosyst. Environ. 88: 11-22.
Finnan, J. M.; Jones, M. B.; Burke, J. I. (1996) A time-concentration study on the effects of ozone on spring wheat (*Triticum aestivum* L)1 Effects on yield. Agric. Ecosyst. Environ. 57: 159-167.
Finnan, J. M.; Jones, M. B.; Burke, J. I. (1996) A time-concentration study on the effects of ozone on spring wheat (*Triticum aestivum* L) 2 A comparison of indices. Agric. Ecosyst. Environ. 57: 169-177.
Fiscus, E. L.; Reid, C. D.; Miller, J. E.; Heagle, A. S. (1997) Elevated CO_2 reduces O_3 flux and O_3-induced yield losses in soybeans: possible implications for elevated CO_2 studies. J. Exp. Bot. 48: 307-313.
Fiscus, E. L.; Miller, J. E.; Booker, F. L.; Heagle, A. S.; Reid, C. D. (2002) The impact of ozone and other limitations on the crop productivity response to CO_2. Technology 8: 181-192.
Fiscus, E. L.; Booker, F. L.; Burkey, K. O. (2005) Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. Plant Cell Environ. 28: 997-1011.
Fishman, J.; Watson, C. E.; Larsen, J. C.; Logan, J. A. (1990) Distribution of tropospheric ozone determined from satellite data. J. Geophys. Res. 95: 3617.
Flagler, R. B. (1998) Recognition of air pollution injury to vegetation: A pictorial atlas; second edition. Pittsburgh, PA: Air & Waste Management Association.
Flückiger, W.; Braun, S.; Hiltbrunner, E. (2002) Effects of air pollutants on biotic stress. In: Bell, J. N. B.; Treshow, M., eds. Air Pollution and Plant Life. John Wiley & Sons Ltd.; pp. 379-406.
Fogel, R.; Cromack, K., Jr. (1977) Effect of habitat and substrate quality on Douglas fir litter decomposition in western Oregon. Can. J. Bot. 55: 1632-1640.
Fontan, J. A.; Minga, A.; Lopez, A.; Druilhet, A. (1992) Vertical ozone profiles in a pine forest. Atmos. Environ. Part A 26A: 863-869.
Foot, J. P.; Caporn, S. J. M.; Lee, J. A.; Ashenden, T. W. (1996) The effect of long-term ozone fumigation on the growth, physiology and frost sensitivity of *Calluna vulgaris*. New Phytol. 133: 505-511.
Foot, J. P.; Caporn, S. J. M.; Lee, J. A.; Ashenden, T. W. (1997) Evidence that ozone exposure increases the susceptibility of plants to natural frosting episodes. New Phytol. 135: 369-374.
Fortin, M.; Maufette, Y.; Albert, P. J. (1997) The effects of ozone-exposed sugar maple seedlings on the biological performance and the feeding preference of the forest tent catepillar (*Malacosoma disstria* Hbn). Environ. Pollut. 97: 303-309.
Fowler, D.; Cape, J. N. (1982) Air pollutants in agriculture and horticulture. In: Unsworth, M. H.; Ormrod, D. P., eds. Effects of gaseous air pollution in agriculture and horticulture. London, United Kingdom: Butterworth Scientific; pp. 3-26.
Fowler, D.; Cape, J. N.; Unsworth, M. H. (1989) Deposition of atmospheric pollutants on forests. Philos. Trans. R. Soc. London B 324: 247-265.
Fox, S.; Mickler, R. A. (1996) Impact of air pollutants on southern pine forests. New York, NY: Springer-Verlag (Lange, O. L.; Mooney, H. A., eds. Ecological studies: v. 118).
Franzaring, J.; Dueck, T. A.; Tonneijck, A. E. G. (2002) Response of two wet grassland species to ozone and water stress. In: Background paper: semi-natural vegetation, workshop establishing ozone critical levels II; November; Goteborg, Sweden. Goteborg, Sweden: Göteborg University, IVL Swedish Environmental Research Institute; pp. 44-49.
Frederick, P. E.; Heath, R. L. (1970) Ozone-induced fatty acid and viability changes in *chlorella*. Plant Physiol. 55: 15-19.

Fredericksen, T. S.; Joyce, B. J.; Skelly, J. M.; Steiner, K. C.; Kolb, T. E.; Kouterick, K. B.; Savage, J. E.; Snyder, K. R. (1995) Physiology, morphology, and ozone uptake of leaves of black cherry seedlings, saplings, and canopy trees. Environ. Pollut. 89: 273-283.
Fredericksen, T. S.; Kolb, T. E.; Skelly, J. M.; Steiner, K. C.; Joyce, B. J.; Savage, J. E. (1996a) Light environment alters ozone uptake per net photosynthetic rate in black cherry trees. Tree Physiol. 16: 485-490.
Fredericksen, T. S.; Skelly, J. M.; Snyder, K. R.; Steiner, K. C. (1996b) Predicting ozone uptake from meteorological and environmental variables. J. Air Waste Manage. Assoc. 46: 464-469.
Fredericksen, T. S.; Skelly, J. M.; Steiner, K. C.; KoIb, T. E.; Kouterick, K. B. (1996c) Size-mediated foliar response to ozone in black cherry trees. Environ. Pollut. 91: 53-63.
Freer-Smith, P. H.; Taylor, G. (1992) Comparative evaluation of the effects of gaseous pollutants, acidic deposition and mineral deficiencies on gas exchange of trees. Agric. Ecosyst. Environ. 42: 321-332.
Friedrich, L.; Moyer, M.; Ward, E.; Ryals, J. (1991) Pathogenesis-related protein 4 is structurally homologous to the carboxy-terminal domains of hevein, Win-1 and Win-2. Mol. Genet. Genom. 230: 113-119.
Froehlich, J. E.; Itoh, A.; Howe, G. A. (2001) Tomato allene oxide synthase and fatty acid hydroperoxide lyase, two cytochrome P450s involved in oxylipin metabolism, are targeted to different membranes of chloroplast envelope. Plant Physiol. 125: 306-317.
Fuentes, J. D.; Gillespie, T. J. (1992) A gas exchange system to study the effects of leaf surface wetness on the deposition of ozone. Atmos. Environ. Part A 26: 1165-1173.
Fuentes, J. D.; Gillespie, T. J.; Den Hartog, G.; Neumann, H. H. (1992) Ozone deposition onto a deciduous forest during dry and wet conditions. Agric. For. Meteorol. 62: 1-18.
Fuentes, J. D.; Hayden, B. P.; Garstang, M.; Lerdau, M.; Fitzjarrald, D.; Baldocchi, D. D.; Monson, R.; Lamb, B.; Geron, C. (2001) New directions: VOCs and biosphere-atmosphere feedbacks. Atmos. Environ. 35: 189-191.
Fuhrer, J. H. (1994) Effects of ozone on managed pasture. 1. Effects of open-top chambers on microclimate, ozone flux and plant growth. Environ. Pollut. 86: 297-305.
Fuhrer, J.; Grimm, A. G.; Tschannen, W.; Shariat-Madari, H. (1992) The response of spring wheat (*Triticum aestivum* L) to ozone at higher elevations: II changes in yield, yield components and grain quality in response to ozone flux. New Phytol. 121: 211-219.
Fuhrer, J.; Shariat-Madari, H.; Perler, R.; Tschannen, W.; Grub, A. (1994) Effects of ozone on managed pasture: II Yield, species composition, canopy structure, and forage quality. Environ. Pollut. 86: 307-314.
Fuhrer, J.; Skärby, L.; Ashmore, M. R. (1997) Critical levels for ozone effects on vegetation in Europe. Environ. Pollution 97: 91-106.
Fujita, E. M.; Croes, B. E.; Bennett, C. L.; Lawson, D. R.; Lurmann, F. W.; Main, H. H. (1992) Comparison of emission inventory and ambient concentration ratios of CO, NMOG, and NO_x in California's south coast air basin. J. Air Waste Manage. Assoc. 42: 264-276.
Fumagalli, I.; Mignanego, L.; Violini, G. (1997) Effects of tropospheric ozone on white clover plants exposed in open-top chambers or protected by the antioxidant ethylene-diurea (EDU). Agronomie 17: 271-281.
Fumagalli, I.; Ambrogi, R.; Mignanego, L. (2001) Yield responses of plants exposed to ambient ozone in the river Po valley (Italy). Agronomie 21: 227-233.
Garcia, P.; Dixon, B.; Mjelde, J.; Adams, R. (1986) Measuring the benefits of environmental change using a duality approach: the case of ozone and Illinois cash grain farms. J. Environ. Econ. Manage. 13: 69-80.
Garten, C. T., Jr.; Hanson, P. J. (1990) Foliar retention of ^{15}N-nitrate and ^{15}N-ammonium by red maple (*Acer rubrum*) and white oak (*Quercus alba*) leaves from simulated rain. Environ. Exp. Bot. 30: 333-342.
Gasper, T.; Penel, C.; Castillo, F. J.; Greppin, H. (1985) A two step control of basic and acidic peroxidases and its significance for growth and development. Physiol. Plant. 64: 418-423.
Gate, I. M.; McNeill, S.; Ashmore, M. R. (1995) Effects of air pollution on the searching behaviour of an insect parasitoid. Water Air Soil Pollut. 85: 1425-1430.
Gates, D. M. (1968) Transpiration and leaf temperature. Annu. Rev. Plant Physiol. 19: 211-238.
Gaucher, C.; Costanzo, N.; Afif, D.; Mauffette, Y.; Chevrier, N.; Dizengremel, P. (2003) The impact of elevated ozone and carbon dioxide on young *Acer saccharum* seedlings. Physiol. Plant. 117: 392-402.
Gelang, J.; Pleijel, H.; Sild, E.; Danielsson, H.; Younis, S.; Selldén, G. (2000) Rate and duration of grain filling in relation to flag leaf senescence and grain yield in spring wheat (*Triticum aestivum*) exposed to different concentrations of ozone. Physiol. Plant. 110: 366-375.
Gerant, D.; Podor, M.; Grieu, P.; Afif, D.; Cornu, S.; Morabito, D.; Banvoy, J.; Robin, C.; Dizengremel, P. (1996) Carbon metabolism enzyme activities and carbon partitioning in *Pinus halepensis* mill Exposed to mild drought and ozone. J. Plant Physiol. 148: 142-147.

Gerosa, G.; Marzuoli, R.; Bussotti, F.; Pancrazi, M.; Ballarin-Denti, A. (2003) Ozone sensitivity of *Fagus sylvatica* and *Fraxinus excelsior* young trees in relation to leaf structure and foliar ozone uptake. Environ. Pollut. 125: 91-98.

Gerosa, G.; Marzuoli, S.; Cieslik, S.; Ballarin-Denti, A. (2004) Stomatal ozone fluxes over a barley field in Italy "Effective exposure" as a possible link between exposure- and flux-based approaches. Atmos. Environ. 38: 2421-2432.

Ghosh, S.; Skelly, J. M.; Innes, J. L.; Skelly, L. (1998) Temporal development of visual injury on the foliage of *Prunus serotina*–a statistical evaluation. Environ. Pollut. 102: 287-300.

Gifford, R. M.; Morison, J. I. L. (1993) Crop responses to the global increase in atmospheric carbon dioxide concentration. In: Buxton, D. R., ed. International crop science I. Madison, WI: Crop Science Society of America; pp. 325-331.

Gillespie, C. T.; Winner, W. E. (1989) Development of lines of radish differing in resistance to O_3 and SO2. New Phytol. 112: 353-361.

Gimeno, B. S.; Bermejo, V.; Reinert, R. A.; Zheng, Y.; Barnes, J. D. (1999) Adverse effects of ambient ozone on watermelon yield and physiology at a rural site in eastern Spain. New Phytol. 144: 245-260.

Goldstein, A. H.; Kurpius, M. R.; Panek, J. A. (2003) Ozone uptake by ponderosa pine in the Sierra Nevada: a measurement perspective. In: Bytnerowicz, A.; Arbaugh, M. J.; Alonso, R., eds. Ozone air pollution in the Sierra Nevada: distribution and effects on forests; pp. distribution and effects on forests; pp. 83-109.

Goodyear, S. N.; Ormrod, D. P. (1988) Tomato response to concurrent and sequential NO_2 and O_3 exposures. Environ. Pollut. 51: 315-326.

Gordon, J. C.; Larson, P. R. (1970) Redistribution of ^{14}C-labeled reserve food in young red pines during shoot elongation. For. Sci. 16: 14-20.

Gorissen, A.; Van Veen, J. A. (1988) Temporary disturbance of translocation of assimilates in Douglas firs caused by low levels of ozone and sulfur dioxide. Plant Physiol. 88: 559-563.

Gorissen, A.; Joosten, N. N.; Jansen, A. E. (1991a) Effects of ozone and ammonium sulphate on carbon partitioning to mycorrhizal roots of juvenile Douglas fir. New Phytol. 119: 243-250.

Gorissen, A.; Schelling, G. C.; Van Veen, J. A. (1991b) Concentration-dependent effects of ozone on translocation of assimilates in Douglas fir. J. Environ. Qual. 20: 169-173.

Gorissen, A.; Joosten, N. N.; Smeulders, S. M.; Van Veen, J. A. (1994) Effects of short-term ozone exposure and soil water availability on the carbon economy of juvenile Douglas-fir. Tree Physiol. 14: 647-657.

Goulder, L. H.; Kennedy, D. (1997) Valuing ecosystem services: philosophical bases and empirical methods. In: Daily, G. C., ed. Nature's services: societal dependence on natural ecosystems. Washington, DC: Island Press; pp. 23-47.

Gower, S. T.; Reich, P. B.; Son, Y. (1993) Canopy dynamics and aboveground production of five tree species with different leaf longevities. Tree Physiol. 12: 327-345.

Grams, T. E. E.; Anegg, S.; Haberle, K. H.; Langebartels, C.; Matyssek, R. (1999) Interactions of chronic exposure to elevated CO_2 and O_3 levels in the phytosynthetic light and dark reactions of European beech (*Fagus sylvatica*). New Phytol. 144: 95-107.

Grantz, D. A.; Farrar, J. F. (1999) Acute exposure to ozone inhibits rapid carbon translocation from source leaves of Pima cotton. J. Exp. Bot. 50: 1253-1262.

Grantz, D. A.; Farrar, J. F. (2000) Ozone inhibits phloem loading from a transport pool: compartmental efflux analysis in Pima cotton. Aust. J. Plant Physiol. 27: 859-868.

Grantz, D. A.; Yang, S. (2000) Ozone impacts on allometry and root hydraulic conductance are not mediated by source limitation nor developmental age. J. Exp. Bot. 51: 919-927.

Grantz, D. A.; Zhang, X. J.; Massman, W. J.; Den Hartog, G.; Neumann, H. H.; Pederson, J. R. (1995) Effects of stomatal conductance and surface wetness on ozone deposition in field-grown grape. Atmos. Environ. 29: 3189-3198.

Grantz, D. A.; Zhang, X. J.; Massman, W.; Delany, A.; Pederson, R. (1997) Ozone deposition to a cotton (*Gossypium hirsutum* L) field: stomatal and surface wetness effects during the California Ozone Deposition experiment. Agric. For. Meteorol. 85: 19-31.

Graumlich, L. J. (1993) A 1000-year record of temperature and precipitation in the Sierra Nevada. Quaternary Res. 39: 249-255.

Greenwood, P.; Greenhalgh, A.; Baker, C.; Unsworth, M. (1982) A computer-controlled system for exposing field crops to gaseous air pollutants. Atmos. Environ. 16: 2261-2266.

Gregg, J. W.; Jones, C. G.; Dawson, T. E. (2003) Urbanization effects on tree growth in the vicinity of New York City [letter]. Nature (London, U. K.) 424: 183-187.

Greitner, C. S.; Winner, W. E. (1989) Effects of O_3 on alder photosynthesis and symbiosis with *Frankia*. New Phytol. 111: 647-656.
Greitner, C. S.; Pell, E. J.; Winner, W. E. (1994) Analysis of aspen foliage exposed to multiple stresses: ozone, nitrogen deficiency and drought. New Phytol. 127: 579-589.
Grennfelt, P. (2004) New directions: recent research findings may change ozone control policies. Atmos. Environ. 38: 2215-2216.
Grimes, H. D.; Perkins, K. K.; Boss, W. F. (1983) Ozone degrades into hydroxyl radical under physiological conditions. Plant Physiol. 72: 1016-1020.
Grodzki, W.; McManus, M. S.; Knížek, M.; Meshkova, V.; Mihalciuc, V.; Novotny, J.; Turčani, M.; Slobodyan, Y. (2004) Occurrence of spruce bark beetles in forest stands at different levels of air pollution stress. Environ. Pollut. 130: 73-83.
Grosjean, D.; Sensharma, D. K.; Cass, G. R. (1994) Fading of colorants by atmospheric pollutants: mass spectrometry studies. Sci. Total Environ. 152: 125-134.
Grosjean, D.; Williams, E. L., II; Grosjean, E. (1995) Monitoring ambient ozone with a network of passive samplers: a feasibility study. Environ. Pollut. 88: 267-273.
Gross, G. G.; Janse, C. (1977) Formation of NADH and hydrogen peroxide by cell wall-associated enzymes from forsythia xylem. Z. Pflanzenphysiol. 84: 447-452.
Groth, J. V.; Krupa, S. V. (2000) Crop ecosystem responses to climatic change: interactive effects of ozone, ultraviolet-B radiation, sulphur dioxide and carbon dioxide on crops. In: Reddy, K. R.; Hodges, H. F., eds. Climate change and global crop productivity. New York, NY: CABI Publishing; pp. 387-405.
Grulke, N. E. (1999) Physiological responses of ponderosa pine to gradients of environmental stressors. In: Miller, P. R.; McBride, J. R., eds. Oxidant Air Pollution Impacts in the Montane Forests of Southern California. Springer-Verlag; pp. 126-163.
Grulke, N. E.; Balduman, L. (1999) Deciduous conifers: high N deposition and O_3 exposure effects on growth and biomass allocation in ponderosa pine. Water Air Soil Pollut. 116: 235-248.
Grulke, N. E.; Lee, E. H. (1997) Assessing visible ozone-induced injury in ponderosa pine. Can. J. For. Res. 27: 1658-1668.
Grulke, N. E.; Miller, P. R. (1994) Changes in gas-exchange characteristics during the life-span of giant sequoia: implications for response to current and future concentrations of atmospheric ozone. Tree Physiol. 14: 659-668.
Grulke, N. E.; Retzlaff, W. A. (2001) Changes in physiological attributes of ponderosa pine from seedling to mature tree. Tree Physiol. 21: 275-286.
Grulke, N. E.; Miller, P. R.; Scioli, D. (1996) Response of giant sequoia canopy foliage to elevated concentrations of atmospheric ozone. Tree Physiol. 16: 575-581.
Grulke, N. E.; Andersen, C. P.; Fenn, M. E.; Miller, P. R. (1998a) Ozone exposure and nitrogen deposition lowers root biomass of ponderosa pine in the San Bernardino Mountains, California. Environ. Pollut. 103: 63-73.
Grulke, N. E.; Miller, P. R.; Leininger, T. D. (1998b) Effect of ozone exposure on seasonal gas exchange of five western conifers. In: Bytnerowicz, A.; Arbaugh, M. J.; Schilling, S. L., eds. Proceedings of the International Symposium on Air Pollution and Climate Change Effects on Forest Ecosystems; February, 1996; Riverside, CA. Albany, CA: USDA Forest Service, Pacific Southwest General Technical Report no. PSW-GTR 166; pp. 229-238.
Grulke, N. E.; Andersen, C. P.; Hogsett, W. E. (2001) Seasonal changes in above- and belowground carbohydrate concentrations of ponderosa pine along a pollution gradient. Tree Physiol. 21: 173-181.
Grulke, N. E.; Preisler, H. K.; Fan, C. C.; Retzlaff, W. A. (2002a) A statistical approach to estimate O_3 uptake of ponderosa pine in a Mediterranean climate. Environ. Pollut. 119: 163-175.
Grulke, N. E.; Preisler, H. K.; Rose, C.; Kirsch, J.; Balduman, L. (2002b) O_3 uptake and drought stress effects on carbon acquisition of ponderosa pine in natural stands. New Phytol. 154: 621-631.
Grulke, N. E.; Johnson, R.; Esperanza, A.; Jones, D.; Nguyen, T.; Posch, S.; Tausz, M. (2003a) Canopy transpiration of Jeffrey pine in mesic and xeric microsites: O_3 uptake and injury response. Trees (Berlin, Ger.) 17: 292-298.
Grulke, N. E.; Johnson, R.; Monschein, S.; Nikolova, P.; Tausz, M. (2003b) Variation in morphological and biochemical O_3 injury attributes of mature Jeffrey pine within canopies and between microsites. Tree Physiol. 23: 923-929.
Grulke, N. E.; Alonso, R.; Nguyen, T.; Cascio, C.; Dobrowolski, W. (2004) Stomata open at night in pole-sized and mature ponderosa pine: implications for O_3 exposure metrics. Tree Physiol. 24: 1001-1010.

Grünhage, L.; Jäger, H. J. (1994) Influence of the atmospheric conductivity on the ozone exposure of plants under ambient conditions: considerations for establishing ozone standards to protect vegetation. Environ. Pollut. 85: 125-129.
Grünhage, L.; Jäger, H. J. (2003) From critical levels to critical loads for ozone: a discussion of a new experimental and modelling approach for establishing flux-response relationships for agricultural crops and native plant species. Environ. Pollut. 125: 99-110.
Grünhage, L.; Dämmgen, U.; Haenel, H. D.; Jäger, H. J.; Holl, A.; Schmitt, J.; Hanewald, K. (1993) A new potential air-quality criterion derived from vertical flux densities of ozone and from plant response. Angew. Bot. 67: 9-13.
Grünhage, L.; Dämmgen, U.; Haenel, H. D.; Jäger, H. J. (1994) Response of a grassland ecosystem to air pollutants: III--the chemical climate: vertical flux densities of gaseous species in the atmosphere near the ground. Environ. Pollut. 85: 43-49.
Grünhage, L.; Haenel, H. D. (1997) PLATIN (PLant-ATmosphere-INteraction) I: a model of plant-atmosphere interaction for estimating absorbed doses of gaseous air pollutants. Environ. Pollut. 98: 37-50.
Grünhage, L.; Jäger, H. J.; Haenel, H. D.; Hanewald, K.; Krupa, S. (1997) PLATIN (plant-atmosphere interaction) II: co-occurrence of high ambient ozone concentrations and factors limiting plant absorbed dose. Environ. Pollut. 98: 51-60.
Grünhage, L.; Jäger, H. J.; Haenel, H. D.; Löpmeier, F. J.; Hanewald, K. (1999) The European critical levels for ozone: improving their usage. Environ. Pollut. 105: 163-173.
Grünhage, L.; Haenel, H. D.; Jäger, H. J. (2000) The exchange of ozone between vegetation and atmosphere: micrometeorological measurement techniques and models. Environ. Pollut. 109: 373-392.
Grünhage, L.; Krause, G. H. M.; Köllner, B.; Benderc, J.; Weigel, H. J.; Jäger, H. J.; Guderian, R. (2001) A new flux-oriented concept to derive critical levels for ozone to protect vegetation. Environ. Pollut. 111: 355-362.
Grünhage, L.; Krupa, S. V.; Legge, A. H.; Jäger, H. J. (2004) Ambient flux-based critical values of ozone for protecting vegetation: differing spatial scales and uncertainties in risk assessment. Atmos. Environ. 38: 2433-2437.
Grunwald, C.; Endress, A. G. (1985) Foliar sterols in soybeans exposed to chronic levels of ozone. Plant Physiol. 77: 245-247.
Grüters, U.; Fangmeier, A.; Jäger, H. J. (1995) Modelling stomatal responses of spring wheat (*Triticum aestivum0 A.* L cv Turbo) to ozone and different levels of water supply. Environ. Pollut. 87: 141-149.
Guderian, R. (1977) Discussion of the suitability of plant responses as a basis for air pollution control measures. In: Billings, W. D.; Golley, F.; Lange, O. L.; Olson, J. S., eds. Air pollution: phytotoxicity of acidic gases and its significance in air pollution control. Berlin, Federal Republic of Germany: Springer Verlag; pp. 75-97.
Guidi, L.; Tonini, M.; Soldatini, G. F. (2000) Effects of high light and ozone fumigation on photosynthesis in *Phaseolus vulgaris*. Plant Physiol. Biochem. 38: 717-725.
Guidi, L.; Nali, C.; Lorenzini, G.; Filippi, F.; Soladatini, G. E. (2001) Effect of chronic ozone fumigation on the photosynthetic process of poplar clones showing different sensitivity. Environ. Pollut. 113: 245-254.
Gunderson, C. A.; Taylor, G. E., Jr. (1988) Kinetics of inhibition of foliar gas exchange by exogenous ethylene: an ultrasensitive response. New Phytol. 110: 517-524.
Gunderson, C. A.; Taylor, G. E., Jr. (1991) Ethylene directly inhibits foliar gas exchange in *Glycine max*. Plant Physiol. 95: 337-339.
Gunderson, C. A.; Sholtis, J. D.; Wullschleger, S. D.; Tissue, D. T.; Hanson, P. J.; Norby, R. J. (2002) Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment.. Plant Cell Environ. 25: 379-393.
Guzy, M. R.; Heath, R. L. (1993) Responses to ozone of varieties of common bean (*Phaseolus vulgaris* L). New Phytol. 124: 617-625.
Häberle, K. H.; Werner, H.; Fabian, P.; Pretzch, H.; Reiter, I.; Matyssek, R. (1999) 'Free-air' ozone fumigation of mature forest trees: a concept for validitating AOT40 under stand conditions. In: Fuhrer, J.; Achermann, B. eds. Critical levels for ozone: level II: workshop under the Convention on Long-Range Transboundary Air Pollution of the United Nations Economic Commission for Europe (UN/ECE); April; Gerzensee, Switzerland; Berne, Switzerland: Bundesamt fur Umwelt, Wald und Landschaft; pp. 133-137.
Hacour, A.; Craigon, J.; Vandermeiren, K.; Ojanperä, K.; Pleijel, H.; Danielsson, H.; Högy, P.; Finnan, J.; Bindi, M. (2002) CO_2 and ozone effects on canopy development of potato crops across Europe. Eur. J. Agron. 17: 257-272.
Hakala, K.; Mela, T. (1996) The effects of prolonged exposure to elevated temperatures and elevated CO_2 levels on the growth, yield and dry matter partitioning of field-sown meadow fescue. Agric. Food Sci. Finl. 5: 285-298.

Hankamer, B.; Barber, J.; Boekema, E. J. (1997) Structure and membrane organization of photosystem II in green plants. Ann. Rev. Plant Physiol. Mol. Biol. 48: 641-671.
Hanson, P. J.; Garten, C. T., Jr. (1992) Deposition of $H_{15}NO_3$ to white oak, red maple, and loblolly pine foliage: experimental observations and a generalized model. New Phytol. 122: 329-337.
Hanson, P. J.; Samuelson, L. J.; Wullschleger, S. D.; Tabberer, T. A.; Edwards, G. (1994) Seasonal patterns of light-saturated photosynthesis and leaf conductance for mature and seedling *Quercus rubra* L foliage: differential sensitivity to ozone exposure. Tree Physiol. 14: 1351-1366.
Hao, X.; Hale, B. A.; Ormrod, D. P.; Papadopoulos, A. P. (2000) Effects of pre-exposure to ultraviolet-B radiation on responses of tomato (*Lycopersicon esculentum* cv New Yorker) to ozone in ambient and elevated carbon dioxide. Environ. Pollut. 110: 217-224.
Harris, M. J.; Bailey-Serres, J. N. (1994) Ozone effects on gene expression and molecular approaches to breeding for air pollution resistance. In: Basra, A. S., ed. Stress-induced gene expression in plants. Langhorne, PA: Harwood Academic Publishers; pp. 185-207.
Harward, M.; Treshow, M. (1975) Impact of ozone on the growth and reproduction of understorey plants in the Aspen zone of western U.S.A. Environ. Conserv. 2: 17-23.
Hausladen, A.; Madamanchi, N. R.; Fellows, S.; Alscher, R. G.; Amundson, R. G. (1990) Seasonal changes in antioxidants in red spruce as affected by ozone. New Phytol. 115: 447-458.
Haynes, R. W.; Adams, D. M. (1992) Assessing economic impacts of air pollution damage to US forests. Asheville, NC: U.S. Department of Agriculture, Southeastern Forest Experiment Station; general technical report no. SE-75.
Heagle, A. S. (1979) Effects of growth media, fertiliser rate and hour and season of exposure on sensitivity of four soybean cultivars to ozone. Environ. Pollut. 18: 313-322.
Heagle, A. S.; Body, D. E.; Heck, W. W. (1973) An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Qual. 2: 365-368.
Heagle, A. S.; Philbeck, R. B.; Knott, W. M. (1979a) Thresholds for injury, growth, and yield loss caused by ozone on field corn hybrids. Phytopathology 69: 21-26.
Heagle, A. S.; Spencer, S.; Letchworth, M. B. (1979b) Yield response of winter wheat to chronic doses of ozone. Can. J. Bot. 57: 1999-2005.
Heagle, A. S.; Letchworth, M. B.; Mitchell, C. A. (1983) Effects of growth medium and fertilizer rate on the yield response of soybeans exposed to chronic doses of ozone. Phytopathology 73: 134-139.
Heagle, A. S.; Heck, W. W.; Lesser, V. M.; Rawlings, J. O. (1987) Effects of daily ozone exposure duration and concentration fluctuation on yield of tobacco. Phytopathology 77: 856-862.
Heagle, A. S.; Kress, L. W.; Temple, P. J.; Kohut, R. J.; Miller, J. E.; Heggestad, H. E. (1988) Factors influencing ozone dose-yield response relationships in open-top field chamber studies. In: Heck, W. W.; Taylor, O. C.; Tingey, D. T. eds. Assessment of crop loss from air pollutants: proceedings of an international conference; October, 1987; Raleigh, NC; New York, NY: Elsevier Applied Science; pp. 141-179.
Heagle, A. S.; Rebbeck, J.; Shafer, S. R.; Blum, U.; Heck, W. W. (1989) Effects of long-term ozone exposure and soil moisture deficit on growth of a ladino clover-tall fescue pasture. Phytopathology 79: 128-136.
Heagle, A. S.; McLaughlin, M. R.; Miller, J. E.; Joyner, R. L.; Spruill, S. E. (1991) Adaptation of a white clover population to ozone stress. New Phytol. 119: 61-68.
Heagle, A. S.; Miller, J. E.; Rawlings, J. O.; Vozzo, S. F. (1991) Effect of growth stage on soybean response to chronic ozone exposure. J. Environ. Qual. 20: 562-570.
Heagle, A. S.; Miller, J. E.; Sherrill.D.E.; Rawlings, J. O. (1993) Effects of ozone and carbon dioxide mixtures on two clones of white clover. New Phytol. 123: 751-762.
Heagle, A. S.; Brandenburg, R. L.; Burns, J. C.; Miller, J. E. (1994a) Ozone and carbon dioxide effects on spider mites in white clover and peanut. J. Environ. Qual. 23: 1168-1176.
Heagle, A. S.; Miller, J. E.; Sherrill, D. E. (1994b) A white clover system to estimate effects of tropospheric ozone on plants. J. Environ. Qual. 23: 613-621.
Heagle, A. S.; Miller, J. E.; Chevone, B. I.; Dreschel, T. W.; Manning, W. J.; McCool, P. M.; Morrison, C. L.; Neely, G. E.; Rebbeck, J. (1995) Response of a white clover indicator system to tropospheric ozone at eight locations in the United States. Water Air Soil Pollut. 85: 1373-1378.
Heagle, A. S.; Reinert, R. A.; Miller, J. E. (1996) Response of white clover to ozone in different environments. J. Environ. Qual. 25: 273-278.
Heagle, A. S.; Miller, J. E.; Booker, F. L. (1998) Influence of ozone stress on soybean response to carbon dioxide enrichment: I Foliar properties. Crop Sci. 38: 113-121.

Heagle, A. S.; Miller, J. E.; Pursley, W. A. (1998) Influence of ozone stress on soybean response to carbon dioxide enrichment: III Yield and seed quality. Crop Sci. 38: 128-134.
Heagle, A. S.; Booker, F. L.; Miller, J. E.; Fiscus, E. L.; Pursley, W. A.; Stefanski, L. A. (1999a) Influence of daily CO_2 exposure duration and root environment on soybean resonse to elevated CO_2. J. Environ. Qual. 28: 666-675.
Heagle, A. S.; Miller, J. E.; Booker, F. L. (1999b) Ozone stress, carbon dioxide enrichment, and nitrogen fertility interactions in cotton. Crop Sci. 39: 731-741.
Heagle, A. S.; Miller, J. E.; Pursley, W. A. (2000) Growth and yield responses of winter wheat to mixtures of ozone and carbon dioxide. Crop Sci. 40: 1656-1664.
Heagle, A. S.; Stefanski, L. A. (2000) Relationships between ambient ozone regimes and white clover forage production using different ozone exposure indexes. Atmos. Environ. 34: 735-744.
Heagle, A. S.; Miller, J. E.; Burkey, K. O.; Eason, G.; Pursley, W. A. (2002) Growth and yield responses of snap bean to mixtures of carbon dioxide and ozone. J. Environ. Qual. 31: 2008-2014.
Heagle, A. S.; Miller, J. E.; Pursley, W. A. (2003) Growth and yield responses of potato to mixtures of carbon dioxide and ozone. J. Environ. Qual. 32: 1603-1610.
Heath, R. L. (1980) Initial events in injury to plants by air pollutants. Annu. Rev. Plant Physiol. 31: 395-431.
Heath, R. L. (1984) Air pollutant effects on biochemicals derived from metabolism: organic, fatty and amino acids. In: Koziol, M. J.; Whatley, F. R., eds. Gaseous Air Pollutants and Plant Metabolism. London, United Kingdom: Butterworths; pp. 275-290.
Heath, R. L. (1987) The biochemistry of ozone attack on the plasma membrane of plant cells. Recent Adv. Phytochem. 21: 29-54.
Heath, R. L. (1988) Biochemical mechanisms of pollutant stress. In: Heck, W. W. ; Taylor, O. C. ; Tingey, D. T. , eds. Assessment of crop loss from air pollutants: proceedings of an international conference; October, 1987; Raleigh, NC. London, United Kingdom: Elsevier Applied Science; pp. 259-286.
Heath, R. L. (1994a) Alterations of plant metabolism by ozone exposure. In: Alscher, R. G.; Wellburn, A. R., eds. Plant Responses to the Gaseous Environment: molecular, metabolic, and physiological aspects. molecular, metabolic, and physiological aspects. London, United Kingdom: Chapman & Hall; pp. 121-146.
Heath, R. L. (1994b) Possible mechanisms for the inhibition of photosynthesis by ozone. Photosynt. Res. 39: 439-451.
Heath, R. L. (1996) The modification of photosynthetic capacity induced by ozone exposure. In: Baker, N. R., ed. Photosynthesis and the environment. Kluwer Academic Publishers; pp. 409-433.
Heath, R. L.; Castillo, F. J. (1987) Membrane disturbances in response to air pollutants. In: Schulte-Hostede, S.; Darrall, N. M.; Blank, L. W.; Wellburn, A. R., eds. Air pollution and plant metabolism. New York, NY: Elsevier Applied Science; pp. 55-75.
Heath, R. L.; Taylor, G. E., Jr. (1997) Physiological processes and plant responses to ozone exposure. In: Sandermann, H.; Wellburn, A. R.; Heath, R. L., eds. Forest Decline and Ozone: a comparison of controlled chamber and field experiments. New York, NY: Springer-Verlag; pp. 317-368. (Ecological Studies, v. 127).
Heck, W. W.; Cowling, E. B. (1997) The need for a long term cumulative secondary ozone standard - an ecological perspective. EM (January): 23-33.
Heck, W. W.; Philbeck, R. B.; Dunning, J. A. (1978) A continuous stirred tank reactor (CSTR) system for exposing plants to gaseous air contaminants. Principles, specifications, construction, and operation. Washington, DC: U.S. Department of Agriculture, Agricultural Research Service; ARS-S-81.
Heck, W. W.; Taylor, O. C.; Adams, R.; Bingham, G.; Miller, J.; Preston, E.; Weinstein, L. (1982) Assessment of crop loss from ozone. J. Air Pollut. Control Assoc. 32: 353-361.
Heck, W. W.; Heagle, A. S.; Miller, J. E.; Rawlings, J. O. (1991) A national program (NCLAN) to assess the impact of ozone on agricultural resources. In: Berglund, R. L.; Lawson, D. R.; McKee, D. J., eds. Tropospheric ozone and the environment: papers from an international conference; March 1990; Los Angeles, CA. Pittsburgh, PA: Air & Waste Management Association; pp. 225-254. (A&WMA transactions series no. TR-19).
Heck, W. W.; Furiness, C. S.; Cowling, E. B.; Sims, C. K. (1998) Effects of ozone on crop, forest, and natural ecosystems: assessment of research needs. EM (October): 11-22.
Heggestad, H. E. (1988) Reduction in soybean seed yields by ozone air pollution? JAPCA 38: 1040-1041.
Heliövaara, K.; Väisänen, R. (1993) Insects and pollution. Boca Raton, FL: CRC Press.
Hendrey, G. R.; Kimball, B. A. (1994) The FACE program. Agric. For. Meteorol. 70: 3-14.
Hendrey, G. R.; Ellsworth, D. S.; Lewin, K. F.; Nagy, J. (1999) A free-air enrichment system for exposing tall forest trees to elevated atmospheric CO_2. Global Change Biol. 5: 293-309.

Hertstein, U.; Fangmeier, A.; Jäger, H. J. (1996) ESPACE-wheat (European stress physiology and climate experiment - Project 1: wheat): objectives, general approach, and first results. J. Appl. Bot. 70: 172-180.
Hertstein, U.; CoIls, J.; Ewert, F.; Van Oijen, M. (1999) Climatic conditions and concentrations of carbon dioxide and air pollutants during 'ESPACE--wheat' experiments. Eur. J. Agron. 10: 163-169.
Hildebrand, E.; Skelly, J. M.; Fredericksen, T. S. (1996) Foliar response of ozone-sensitive hardwood tree species from 1991 to 1993 in the Shenandoah National Park, Virginia. Can. J. For. Res. 26: 658-669.
Ho, I.; Trappe, J. M. (1984) Effects of ozone exposure on mycorrhiza formation and growth of *Festuca arundinacea*. Environ. Exp. Bot. 24: 71-74.
Hofstra, G.; Ali, A.; Wukasch, R. T.; Fletcher, R. A. (1981) The rapid inhibition of root respiration after exposure of bean (*Phaseolus vulgaris* L) plants to ozone. Atmos. Environ. 15: 483-487.
Hogsett, W. E.; Plocher, M.; Wildman, V.; Tingey, D. T.; Bennett, J. P. (1985a) Growth response of two varieties of slash pine seedlings to chronic ozone exposures. Can. J. Bot. 63: 2369-2376.
Hogsett, W. E.; Tingey, D. T.; Holman, S. R. (1985b) A programmable exposure control system for determination of the effects of pollutant exposure regimes on plant growth. Atmos. Environ. 19: 1135-1145.
Hogsett, W. E.; Olszyk, D.; Ormrod, D. P.; Taylor, G. E., Jr.; Tingey, D. T. (1987a) Air pollution exposure systems and experimental protocols: volume 1: a review and evaluation of performance. Corvallis, OR: U.S. Environmental Protection Agency, Environmental Research Laboratory; EPA report no. EPA/600/3-87/037a. Available from: NTIS, Springfield, VA; PB88-181680.
Hogsett, W. E.; Olszyk, D.; Ormrod, D. P.; Taylor, G. E., Jr.; Tingey, D. T. (1987b) Air pollution exposure systems and experimental protocols: volume 2: description of facilities. Corvallis, OR: U.S. Environmental Protection Agency, Environmental Research Laboratory; EPA report no. EPA/600/3-87/037b. Available from: NTIS, Springfield, VA; PB88-181698.
Hogsett, W. E.; Tingey, D. T.; Lee, E. H. (1988) Ozone exposure indices: concepts for development and evaluation of their use. In: Heck, W. W.; Taylor, O. C.; Tingey, D. T. eds. Assessment of crop loss from air pollutants: proceedings of an international conference; October 1987; Raleigh, NC; New York, NY: Elsevier Applied Science; pp. 107-138.
Hogsett, W. E.; Tingey, D. T.; Hendricks, C.; Rossi, D. (1989) Sensitivity of western conifers to SO2 and seasonal interaction of acid fog and ozone. In: Olson, R. K.; Lefohn, A. S., eds. Effects of air pollution on western forests [an A&WMA symposium; June; Anaheim, CA]. Air Pollution Control Association; pp. 469-491 (APCA transactions series: no. 16).
Hogsett, W. E.; Herstrom, A. A.; Laurence, J. A.; Lee, E. H.; Weber, J. E.; Tingey, D. T. (1995) Risk characterization of tropospheric ozone to forests. In: Lee, S. D.; Schneider, T. eds. Comparative risk analysis and priority setting for air pollution issues: proceedings of the 4th U.S.-Dutch international symposium; June 1993; Keystone, CO; Pittsburgh, PA: Air & Waste Management Association; pp. 119-145. (AWMA publication no. VIP-43).
Hogsett, W. E.; Weber, J. E.; Tingey, D.; Herstrom, A.; Lee, E. H.; Laurence, J. A. (1997) Environmental auditing: an approach for characterizing tropospheric ozone risk to forests. Environ. Manage. 21: 105-120.
Holland, D. M.; Saltzman, N.; Cox, L. H.; Nychka, D. (1999) Spatial prediction of sulfur dioxide in the eastern United States. In: Gómez-Hernández, J.; Soares, A.; Froidevaux, R. eds. geoENV II: geostatistics for environmental applications, proceedings of the second European conference; November 1998; Valencia, Spain; Dordrecht, The Netherlands: Kluwer Academic Publishers; pp. 65-76. (Quantitative geology and geostatistics: v. 10).
Holopainen, J. K.; Kainulainen, P.; Oksanen, J. (1997) Growth and reproduction of aphids and levels of free amino acids in Scots pine and Norway spruce in an open-air fumigation with ozone. Global Change Biol. 3: 139-147.
Holton, M. K.; Lindroth, R. L.; Nordheim, E. V. (2003) Foliar quality influences tree-herbivore-parisitoid interactions: effects of elevated CO_2, O_3, and plant genotype. Oecologia 137: 233-244.
Horemans, N.; Foyer, C. H.; Asard, H. (2000) Transport and action of ascorbate at the plant plasma membrane. Trends Plant Sci. 5: 263-267.
Hormaza, J. I.; Pinney, K.; Polito, V. S. (1996) Correlation in the tolerance to ozone between sporophytes and male gametophytes of several fruit and tree nut species (Rosaceae). Sex. Plant Reprod. 9: 44-48.
Houpis, J. L. J.; Costella, M. P.; Cowles, S. (1991) A branch exposure chamber for fumigating ponderosa pine to atmospheric pollution. J. Environ. Qual. 20: 467-474.
Howe, G. A.; Lee, G. I.; Itoh, A.; Li, L.; DeRocher, A. (2000) Cytochrome P450-dependent metabolism of oxylipins in tomato: cloning and expression of allene oxide synthase and fatty acid hydroperoxide lyase. Plant Physiol. 123: 711-724.

Hui, D.; Sims, D. A.; Johnson, D. W.; Chang, W.; Luo, Y. (2002) Effects of gradual versus step increases in carbon dioxide on *Plantago* photosynthesis and growth in a microcosm study. Environ. Exp. Bot. 47: 51-66.
Hummel, R. L.; Brandenburg, R. L.; Heagle, A. S.; Arellano, C. (1998) Effects of ozone on reproduction of two-spotted spider mite (Acari: Tetrannychidae) on white clover. Environ. Entomol. 27: 388-394.
Huttunen, S.; Heikkilä, H.; Bucher, J.-B.; Sundberg, B.; Jarvis, P. G.; Matyssek, R. (2001) Trends in European forest tree physiological research. Amsterdam, The Netherlands: Kluwer, 262 pp, 262.
Idso, K. E.; Idso, S. B. (1994) Plant responses to atmospheric CO_2 enrichment in the face of environmental constraints: a review of the past 10 years' research. Agric. For. Meteorol. 69: 153-203.
Innes, J. L.; Skelly, J. M.; Schaub, M. (2001) Ozone and broadleaved species A guide to the identification of ozone-induced foliar injury. Bern, Switzerland: Paul Haupt Publishers.
Intergovernmental Panel on Climate Change (IPCC) (1996) Climate change 1995: the science of climate change: contribution of working group I to the second assessment of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom: Cambridge University Press.
Intergovernmental Panel on Climate Change (IPCC) (2001) Climate change 2001: the scientific basis Contribution of working group I to the third assessment report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom: Cambridge University Press.
Isebrands, J. G.; Dickson, R. E.; Rebbeck, J.; Karnosky, D. F. (2000) Interacting effects of multiple stresses on growth and physiological processes in northern forest trees. In: Mickler, R. A.; Birsdey, R. A.; Hom, J., eds. Responses of northern U.S. forests to environmental change. New York, NY: Springer-Verlag; pp. 149-180. (Ecological studies: v. 139).
Isebrands, J. G.; McDonald, E. P.; Kruger, E.; Hendrey, G.; Percy, K.; Pregitzer, K.; Sober, J.; Karnosky, D. F. (2001) Growth responses of *Populus tremuloides* clones to interacting carbon dioxide and tropospheric ozone. Environ. Pollut. 115: 359-371.
Islam, K. R.; Mulchi, C. L.; Ali, A. A. (2000) Interactions of tropospheric CO_2 and O_3 enrichments and moisture variations on microbial biomass and respiration in soil. Global Change Biol. 6: 255-265.
Ito, O.; Okano, K.; Kuroiwa, M.; Totsuka, T. (1985) Effects of NO_2 and O_3 alone or in combination on kidney bean plants (*Phaseolus vulgaris* L): growth, partitioning of assimilates and root activities. J. Exp. Bot. 36: 652-662.
Izuta, T. (1998) Ecopysiological responses of Japanese forest tree species to ozone, simulated acid rain and soil acidification. J. Plant Res. 111: 471-480.
Jackson, G. E. (1995) The effect of ozone, nitrogen dioxide and nitric oxide fumigation of cereals on the rose grain aphid *Metopolophium dirhodum*. Agric. Ecosyst. Environ. 54: 187-194.
Jackson, D. M.; Heagle, A. S.; Eckel, R. V. W. (1999) Ovipositional response of tobacco hornworm moths (Lepidoptera: Sphyngidae) to tobacco plants grown under elevated levels of ozone. Environ. Entomol. 28: 566-571.
Jackson, D. M.; Rufty, T. W.; Heagle, A. S.; Severson, R. F.; Eckel, R. W. V. (2000) Survival and development of tobacco hornworm larvae on tobacco plants grown under elevated levels of ozone. J. Chem. Ecol. 26: 1-18.
Jacobson, J. S. (1977) The effects of photochemical oxidants on vegetation. In: Ozon und Begleitsubstanzen im photochemischen Smog: das Kolloquium [Ozone and related substances in photochemical smog: the colloquium]; September 1976; Düsseldorf, Federal Republic of Germany; Dusseldorf, Federal Republic of Germany: VDI-Verlag GmbH; pp. 163-173. (VDI-Berichte nr. 270).
Jäger, H. J.; Herstein, U.; Fangmeier, A. (1999) The European Stress Physiology and Climate Experiment - Project 1: wheat (ESPACE-wheat); introduction, aims and methodology. Eur. J. Agron. 10: 155-162.
James, R. L.; Cobb, F. W., Jr.; Miller, P. R.; Parmeter, J. R., Jr. (1980) Effects of oxidant air pollution on susceptibility of pine roots to *Fomes annosus*. Phytopathology 70: 560-563.
Jans, U.; Hoigné, J. (2000) Atmospheric water: transformation of ozone into OH-radicals by sensitized photoreactions or black carbon. Atmos. Environ. 34: 1069-1085.
Janson, R.; Granat, L. (1999) A foliar rinse study of the dry deposition of nitric acid to a coniferous forest. Agric. For. Meteorol. 98-99: 683-696.
Jarvis, P. G. (1976) The interpretation of the variation in leaf water potential and stomatal conductance found in canopies in the field. Philosoph. Trans. Royal Soc. London Series B - Biological Sciences 273: 593-610.
Jiang, C. Z.; Rodermel, S. R.; Shibles, R. M. (1993) Photosynthesis, rubisco activity and amount, and their regulation by transcription in senescing soybean leaves. Plant Physiol. 101: 105-112.
Jiménez, A.; Calvo, E.; Martín, C.; Porcuna, J. L.; Sanz, M. J. (2001) Estudio de la interacción entre el ozone y el desarrolio de las virosis en el cultivo del tomate. Agricola Vergel 231: 141-150.
Johnson, D. W.; Taylor, G. E. (1989) Role of air pollution in forest decline in eastern North America. Water Air Soil Pollut. 48: 21-43.

Johnson, B. G.; Hale, B. A.; Ormrod, D. P. (1996a) Carbon dioxide and ozone effects on growth of a legume-grass mixture. J. Environ. Qual. 25: 908-916.
Johnson, J. D.; Chappelka, A. H.; Hain, F. P.; Heagle, A. S. (1996b) Interactive effects of air pollutants with abiotic and biotic factors on southern pine forests. In: Cox, S.; Mickler, R. A., eds. Impact of air pollutants on southern pine forests. Springer; pp. 281-312.
Jones, H. G.; Lacasse, N. L.; Liggett, W. S.; Weatherford, F. (1977) Experimental air exclusion system for field studies of SO_2 effects on crop productivity. Washington, DC: U.S. Environmental Protection Agency; EPA-600/7-77-122.
Jones, C. G.; Coleman, J. S.; Findlay, S. (1994) Effects of ozone on interactions between plants, consumers and decomposers. In: Alscher, R. G.; Wellburn, A. R., eds. Plant responses to the gaseous environment. Chapman and Hall; pp. 339-363.
Joss, U.; Graber, W. K. (1996) Profiles and simulated exchange of H2O, O_3, NO_2 between the atmosphere and the HartX Scots pine plantation. Theor. Appl. Climatol. 53: 157-172.
Kainulainen, P.; Holopainen, J. K.; Hyttinen, H.; Oksanen, J. (1994) Effect of ozone on the biochemistry and aphid infestation of Scots pine. Phytochemistry 35: 39-42.
Kainulainen, P.; Holopainen, J. K.; Holopainen, T. (2000a) Combined effects of ozone and nitrogen on secondary compounds, amino acids, and aphid performance in Scots pine. J. Environ. Qual. 29: 334-342.
Kainulainen, P.; Utriainen, J.; Holopainen, J. K.; Oksanen, J.; Holopainen, T. (2000b) Influence of elevated ozone and limited nitrogen availability on conifer seedlings in an open-air fumigation system: effects on growth, nutrient content, mycorhiza, needle ultrastructure, starch and secondary compounds. Global Change Biol. 6: 345-355.
Kainulainen, P.; Holopainen, T.; Holopainen, J. K. (2003) Decomposition of secondary compounds from needle litter of Scots pine grown under elevated CO_2 and O_3. Glob. Change Biol. 9: 295-304.
Kangasjärvi, J.; Talvinen, J.; Utriainen, M.; Karjalainen, R. (1994) Plant defense systems induced by ozone. Plant Cell Environ. 17: 783-794.
Kanoun, M.; Goulas, P.; Bassères, A.; Biolley, J. P. (2002) Ozone-induced oxidation of Rubisco: from an ELISA quantification of carbonyls to putative pathways leading to oxidizing mechanisms. Funct. Plant Biol. 29: 1357-1363.
Kärenlampi, L.; Skärby, L. (1996) Critical levels for ozone in Europe: testing and finalizing the concepts UN-ECE workshop report. In: Proceedings of UN-ECE convention on long-range transboundary air pollution workshop; April; Kuopio, Finland. Kupio, Finland: University of Kuopio, Department of Ecology and Environmental Science.
Karlsson, P. E.; Medin, E. L.; Wickström, H.; Selldén, G.; Wallin, G.; Ottosson, S.; Skärby, L. (1995) Ozone and drought stress: interactive effects on the growth and physiology of Norway spruce (*Picea abies* (L) Karst). Water Air Soil Pollut. 85: 1325-1330.
Karlsson, P. E.; Medin, E. L.; Wallin, G.; Selldén, G.; Skärby, L. (1997) Effects of ozone and drought stress on the physiology and growth of two clones of Norway spruce (*Picea abies*). New Phytol. 136: 265-275.
Karlsson, P. E.; Pleijel, H.; Karlsson, G. P.; Medin, E. L.; Skärby, L. (2000) Simulations of stomatal conductance and ozone uptake to Norway spruce saplings in open-top chambers. Environ. Pollut. 109: 443-451.
Karlsson, P. E.; Medin, E. L.; Selldén, G.; Wallin, G.; Ottosson, S.; Pleijel, H.; Skärby, L. (2002) Impact of ozone and reduced water supply on the biomass accumulation of Norway spruce saplings. Environ. Pollut. 19: 237-244.
Karlsson, G. P.; Karlsson, P. E.; Danielsson, H.; Pleijel, H. (2003) Clover as a tool for bioindication of phytotoxic ozone–5 years of experience from southern Sweden–consequences for the short-term critical levels. Sci. Total Environ. 301: 205-213.
Karlsson, P. E.; Uddling, J.; Skärby, L.; Wallin, G.; Selldén, G. (2003) Impact of ozone on the growth of birch (*Betula pendula*) saplings. Environ. Pollut. 124: 485-495.
Karlsson, P. E.; Selldén, G.; Pleijel, H. (2003) Establishing ozone critical levels II. Gothenburg, Sweden: IVL Swedish Environmental Research Institute; IVL Report B 1523.
Karlsson, G. P.; Karlsson, P. E.; Soya, G.; Vandermeiren, K.; Pleijel, H. (2004a) Test of the short-term critical levels for acute ozone injury on plants–improvements by ozone uptake modelling and the use of an effect threshold. Atmos. Environ. 38: 2237-2245.
Karlsson, P. E.; Uddling, J.; Braun, S.; Broadmeadow, M.; Elvira, S.; Gimeno, B. S.; Le Thiec, D.; Okansen, E.; Vandermeiren, K.; Wilkinson, M.; Emberson, L. (2004b) New critical levels for ozone effects on young trees based on AOT40 and simulated cumulative leaf uptake of ozone. Atmos. Environ. 38: 2283-2294.

Karnosky, D. F. (1981) Changes in eastern white pine stands related to air pollution stress. Mitt. Forstl. Bundesversuchsanst. Wien 137: 41-45.
Karnosky, D. F. (2003) Impacts of elevated atmospheric CO_2 on forest trees and forest ecosystems: knowledge gaps. Environ. Int. 29: 161-169.
Karnosky, D. F.; Gagnon, Z. E.; Dickson, R. E.; Coleman, M. D.; Lee, E. H.; Isebrands, J. G. (1996) Changes in growth, leaf abscission, biomass associated with seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. Can. J. For. Res. 26: 23-37.
Karnosky, D. F.; Podila, G. K.; Gagnon, Z.; Pechter, P.; Akkapeddi, A.; Coleman, M.; Dickson, R. E.; Isebrands, J. G. (1998) Genetic control of responses to interacting O_3 and CO_2 in *Populus tremuloides*. Chemosphere 36: 807-812.
Karnosky, D. F.; Mankovska, B.; Percy, K.; Dickson, R. E.; Podila, G. K.; Sober, J.; Noormets, A.; Hendrey, G.; Coleman, M. D.; Kubiske, M.; Pregitzer, K. S.; Isebrands, J. G. (1999) Effects of tropospheric ozone on trembling aspen and interaction with CO_2: results from an O_3-gradient and a FACE experiment. Water Air Soil Pollut. 116: 311-322.
Karnosky, D. F.; Gielen, B.; Ceulemans, R.; Schlesinger, W. H.; Norby, R. J.; Oksanen, E.; Matyssek, R.; Hendrey, G. R. (2001) FACE systems for studying the impact of greenhouse gases on forest ecosystems. In: Karnosky, D. F.; Ceulemans, R.; Scarascia-Mugnozza, G. E.; Innes, J. L. eds. The impact of carbon dioxide and other greenhouse gases on forest ecosystems, Report No. 3 of the IUFRO Task Force on Environmental Change; Wallingford, United Kingdom: CABI Publ.; pp. 297-324.
Karnosky, D. F.; Ceulemans, R.; Scarascia-Mugnozza, G. E.; Ines, J. L. (2001a) The impact of carbon dioxide and other greenhouse gases on forest ecosystems: report no 3 of the IUFRO Task Force on Environmental Change. Wallingford, United Kingdom: CABI Publishing; Available: http://www.cabi-publishing.org/Bookshop/Readingroom/0851995519.asp [19 August, 2003].
Karnosky, D. F.; Oksanen, E.; Dickson, R. E.; Isebrands, J. G. (2001b) Impact of interacting greenhouse gases on forest ecosystems. In: Karnosky, D. F.; Ceulemans, R.; Scarascia-Mugnozza, G. E.; Innes, J. L., eds. The impact of carbon dioxide and other greenhouse gases of forest ecosystems: report no. 3 of the IUFRO Task Force on Environmental Change. Wallingford, United Kingdom: CABI Publishing; pp. 253-267 (IUFRO research series no. 8).
Karnosky, D. F.; Percy, K. E.; Xiang, B.; Callan, B.; Noormets, A.; Mankovska, B.; Hopkin, A.; Sôber, J.; Jones, W.; Dickson, R. E.; Isebrands, J. G. (2002) Interacting elevated CO_2 and tropospheric O_3 predisposes aspen (*Populus tremuloides* Michx) to infection by rust (*Melampsora medusae* f sp *tremuloidae*). Global Change Biol. 8: 329-338.
Karnosky, D. F.; Zak, D. R.; Pregitzer, K. S.; Awmack, C. S.; Bockheim, J. G.; Dickson, R. E.; Hendrey, G. R.; Host, G. E.; King, J. S.; Kopper, B. J.; Kruger, E. L.; Kubiske, M. E.; Lindroth, R. L.; Mattson, W. J.; McDonald, E. P.; Noormets, A.; Oksanen, E.; Parsons, W. F. J.; Percy, K. E.; Podila, G. K.; Riemenschneider, D. E.; Sharma, P.; Thakur, R.; Sôber, A.; Sôber, J.; Jones, W. S.; Anttonen, S.; Vapaavuori, E.; Mankovska, B.; Heilman, W.; Isebrands, J. G. (2003a) Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: A synthesis of molecular to ecosystem results from the Aspen FACE project. Funct. Ecol. 17: 289-304.
Karnosky, D. F.; Percy, K.; Mankovska, B.; Pritchard, T.; Noormets, A.; Dickson, R. E.; Jepsen, E.; Isebrands, J. G. (2003b) Ozone affects the fitness of trembling aspen. In: Karnosky, D. F.; Percy, K. E.; Chappelka, A. H.; Simpson, C. J.; Pikkarainen, J., eds. Air pollution, global change and forests in the new millenium. Elsevier Science. (Developments in Environmental Science, 3).
Karnosky, D. F.; Pregitzer, K. S.; Zak, D. R.; Kubiske, M. E.; Hendrey, G. R.; Weinstein, D.; Nosal, M.; Percy, K. E. (2005) Scaling ozone responses of forest trees to the ecosystem level in a changing climate. Plant Cell Environ. 28: 965-981.
Karpinski, S.; Wingsle, G.; Karpinska, B.; Hällgren, J. E. (1993) Molecular response to photooxidative stress in *Pinus sylvestris* (L): II Differential expression of Cu-Zn superoxide dismutases and glutathione reductase. Plant Physiol. 103: 1385-1391.
Kasurinen, A. E.; Helmisaari, H. S.; Holopainen, T. (1999) The influence of elevated CO_2 and O_3 on fine roots and mycorrihizas of naturally growing young Scots pine trees during three exposure years. Global Change Biol. 5: 771-780.
Kats, G.; Thompson, C. R.; Kuby, W. C. (1976) Improved ventilation of open top greenhouses. J. Air Pollut. Control Assoc. 26: 1089-1090.
Kats, G.; Olszyk, D. M.; Thompson, C. R. (1985a) Open top experimental chambers for trees. J. Air Pollut. Control Assoc. 35: 1298-1301.

Kats, G.; Dawson, P. J.; Bytnerowicz, A.; Wolf, J. W.; Thompson, C. R.; Olszyk, D. M. (1985b) Effects of ozone or sulfur dioxide on growth and yield of rice. Agric. Ecosyst. Environ. 14: 103-117.
Kellomäki, S.; Wang, K. Y. (1997a) Effects of elevated O_3 and CO_2 concentrations on photosynthesis and stomatal conductance in Scots pine. Plant Cell Environ. 20: 995-1006.
Kellomäki, S.; Wang, K. Y. (1997b) Effects of elevated O_3 and CO_2 on chlorophyll fluorescence and gas exchange in Scots pine during the third growing season. Environ. Pollut. 97: 17-27.
Kelting, D. L.; Burger, J. A.; Edwards, G. S. (1995) The effects of ozone on the root dynamics of seedlings and mature red oak (*Quercus rubra* L). For. Ecol. Manage. 79: 197-206.
Kerstiens, G.; Lendzian, K. J. (1989) Interaction between ozone and plant cuticles: I ozone deposition and permeability. New Phytol. 112: 13-19.
Khan, M. R.; Khan, M. W. (1994) Single and interactive effects of O_3 and SO_2 on tomato. Environ. Exp. Bot. 34: 461-469.
Khan, M. R.; Khan, M. W. (1997) Effects of the root-knot nematode, *Meloidogyne incognita*, on the sensitivity of tomato to sulfur dioxide and ozone. Environ. Exp. Bot. 38: 117-130.
Khan, M. R.; Khan, M. W. (1998a) Interactive effects of ozone and powdery mildew (*Sphaerotheca fuliginea*) on bottle gourd (*Lagenaria siceraria*). Agric. Ecosyst. Environ. 70: 109-118.
Khan, M. R.; Khan, M. W. (1998b) Interactive effects of ozone and root-knot nematode on tomato. Agric. Ecosyst. Environ. 70: 97-107.
Khan, M. R.; Khan, M. W. (1999) Effects of intermittent ozone exposures on powdery mildew of cucumber. Environ. Exp. Bot. 42: 163-171.
Kickert, R. N.; Krupa, S. V. (1991) Modeling plant response to tropospheric ozone: a critical review. Environ. Pollut. 70: 271-383.
Kickert, R. N.; Tonella, G.; Simonov, A.; Krupa, S. V. (1999) Predictive modeling of effects under global change. Environ. Pollut. 100: 87-132.
Kim, H. J.; Helfand, G. E.; Howitt, R. E. (1998a) An economic analysis of ozone control in California's San Joaquin Valley. J. Agric. Resource Econ. 23: 55-70.
Kim, J. S.; Chappelka, A. H.; Miller-Goodman, M. S. (1998b) Decomposition of blackberry and broomsedge bluestem as influenced by ozone. J. Environ. Qual. 27: 953-960.
King, J. S.; Pregitzer, K. S.; Zak, D. R.; Sober, J.; Isebrands, J. G.; Dickson, R. E.; Hendrey, G. R.; Karnosky, D. F. (2001) Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO_2 and tropospheric O_3. Oecologia 128: 237-250.
Kleier, C.; Farnsworth, B.; Winner, W. (1998) Biomass, reproductive output, and physiological responses of rapid-cycling Brassica (*Brassica rapa*) to ozone and modified root temperature. New Phytol. 139: 657-664.
Kleier, C.; Farnsworth, B.; Winner, W. (2001) Photosynthesis and biomass allocation of radish cv "Cherry Belle" in response to root temperature and ozone. Environ. Pollut. 111: 127-133.
Kliebenstein, D. J.; Monde, R. A.; Last, R. L. (1998) Superoxide dismutase in arabidopsis: an eclectic enzyme family with disparate regulation and protein localization. Plant Physiol. 118: 637-650.
Kobayashi, K.; Okada, M.; Nouchi, I. (1994) A chamber system for exposing rice (*Oryza sativa* L) to ozone in a paddy field. New Phytol. 126: 317-325.
Kobayashi, K.; Okada, M.; Nouchi, I. (1995) Effects of ozone on dry matter partitioning and yield of Japanese cultivars of rice (*Oryza sativa* L). Agric. Ecosyst. Environ. 53: 109-122.
Koch, J. R.; Scherzer, A. J.; Eshita, S. M.; Davis, K. R. (1998) Ozone sensitivity in hybrid poplar is correlated with a lack of defense-gene activation. Plant Physiol. 118: 1243-1252.
Koch, J. R.; Creelman, R. A.; Eshita, S. M.; Seskar, M.; Mullet, J. E.; Davis, K. R. (2000) Ozone sensitivity in hybrid poplar correlates with insensitivity to both salicylic acid and jasmonic acid The role of programmed cell death in lesion formation. Plant Physiol. 123: 487-496.
Kohut, R. J.; Laurence, J. A.; Amundson, R. G. (1988) Effects of ozone and sulfur dioxide on yield of red clover and timothy. J. Environ. Qual. 17: 580-585.
Kohut, R. J.; Laurence, J. A.; King, P.; Raba, R. (2000) Identification of bioindicator species for ozone and assessment of the responses to ozone of native vegetation at Acadia National Park. Denver, CO: U.S. National Park Service, Air Resources Division; Final Report Acadia D-175. Technical Report NPS/BSO-RNR/NRTR/OO-13.
Kolb, T. E.; Fredericksen, T. S.; Steiner, K. C.; Skelly, J. M. (1997) Issues in scaling tree size and age responses to ozone: a review. Environ. Pollut. 98: 195-208.
Kolb, T. E.; Matyssek, R. (2001) Limitations and perspectives about scaling ozone impacts in trees. Environ. Pollut. 115: 373-393.

Köllner, B.; Krause, G. H. M. (2003) Effects of two different ozone exposure regimes on chlorophyll and sucrose content of leaves and yield parameters of sugar beet (*Beta Vulgaris* L.) and rape (*Brassica napus* L.). Water Air Soil Pollut. 144: 317-332.

Kolomiets, M. V.; Chen, H.; Gladon, R. J.; Braun, E. J.; Hannapel, D. J. (2000) A leaf lipoxygenase of potato induced specifically by pathogen infection. Plant Physiol. 124: 1121-1130.

Kopp, R. J.; Vaughn, W. J.; Hazilla, M.; Carson, R. (1985) Implications of environmental policy for US agriculture: the case of ambient ozone standards. J. Environ. Manage. 20: 321-331.

Kopper, B. J.; Lindroth, R. L. (2003a) Responses of trembling aspen (*Populus tremuloides*) phytochemistry and aspen blotch leafminer (*Phyllonorycter tremuloidiella*) performance to elevated levels of atmospheric CO_2 and O_3. Agric. For. Entomol. 5: 17-26.

Kopper, B. J.; Lindroth, R. L. (2003b) Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. Oecologia 134: 95-103.

Kostka-Rick, R.; Manning, W. J. (1992a) Partitioning of biomass and carbohydrates in field-grown radish under ambient concentrations of ozone, and treated with the anti-ozonant ethylene-diurea (EDU). New Phytol. 121: 187-200.

Kostka-Rick, R.; Manning, W. J. (1992b) Effects and interactions of ozone and the anti-ozonant EDU at different stages of radish (*Raphanus sativus* L) development. J. Exp. Bot. 43: 1621-1631.

Kostka-Rick, R.; Manning, W. J. (1993) Dynamics of growth and biomass partitioning in field-grown bush bean (*Phaseolus vulgaris* L), treated with the antiozonant ethylenediurea (EDU). Agric. Ecosyst. Environ. 47: 195-214.

Kostka-Rick, R.; Manning, W. J.; Buonaccorsi, J. P. (1993) Dynamics of biomass partitioning in field-grown radish varieties, treated with ethylenediurea. Environ. Pollut. 80: 133-145.

Kouterick, K. B.; Skelly, J. M.; Fredericksen, T. S.; Steiner, K. C.; KoIb, T. E.; Ferdinand, J. A. (2000) Foliar injury, leaf gas exchange and biomass responses of black cherry (*Prunus serotina* Ehrh) half-sibling families to ozone exposure. Environ. Pollut. 107: 117-126.

Kozioł, M. J.; Whatley, F. R. (1984) Gaseous air pollutants and plant metabolism. London, United Kingdom: Butterworths Scientific.

Kozlowski, T. T.; Pallardy, S. G. (1997) Physiology of woody plants. San Diego, CA: Academic Press.

Kraft, M.; Weigel, H. J.; Mejer, G. J.; Brandes, F. (1996) Reflectance measurements of leaves for detecting visible and non-visible ozone damage to crops. J. Plant Physiol. 148: 148-154.

Krapp, A.; Hofmann, B.; Schäfer, C.; Stitt, M. (1993) Regulation of the expression of *rbc*S and other photosynthetic genes by carbohydrates: a mechanism for the "sink regulation" of photosynthesis? Plant J. 3: 817-828.

Krupa, S. V.; Groth, J. V. (2000) Global climate change and crop responses: uncertainties associated with the current methodologies. In: Agrawal, S. B.; Agrawal, M., eds. Environmental pollution and plant responses. Boca Raton, FL: Lewis Publishers; pp. 1-18.

Krupa, S. V.; Jäger, H. J. (1996) Adverse effects of elevated levels of ultraviolet (UV)-B radiation and ozone (O_3) on crop growth and productivity. In: Bazzaz, F.; Sombroek, W., eds. Global climate change and agricultural production. Chichester, United Kingdom: John Wiley & Sons; pp. 141-169. Available: http://www.fao.org/docrep/W5183E/w5183e00.htm [28 August, 2003].

Krupa, S. V.; Grünhage, L.; Jäger, H. J.; Nosal, M.; Manning, W. J.; Legge, A. H.; Hanewald, K. (1995) Ambient ozone (O_3) and adverse crop response: a unified view of cause and effect. Environ. Pollut. 87: 119-126.

Krupa, S. V.; Kickert, R. N.; Jäger, H. J. (1998) Elevated ultraviolet (UV)-B radiation and agriculture. New York, NY: Springer-Verlag Environmental Intelligence Unit.

Krupa, S. V.; Nosal, M.; Peterson, D. L. (2001) Use of passive ozone O_3 samplers in vegetation effects assessment. Environ. Pollut. 112: 303-309.

Krupa, S.; Nosal, M. (2001) Relationships between passive sampler and continuous ozone (O_3) measurement data in ecological effects research. Scientific World 1: 593-601.

Krupa, S. V.; Nosal, M.; Ferdinand, J. A.; Stevenson, R. E.; Skelly, J. M. (2003) A multi-variate statistical model integrating passive sampler and meteorology data to predict the frequency distributions of hourly ambient ozone (O_3) concentrations. Environ. Pollut. 124: 173-178.

Kuehler, E. A.; Flagler, R. B. (1999) The effects of sodium erythorbate and ethylenediurea on photosynthetic function of ozone-exposed loblolly pine seedlings. Environ. Pollut. 105: 25-35.

Kuik, O. J.; Helming, F. M.; Dorland, C.; Sparinks; F.A. (2000) The economic benefits to agriculture of a low-level ozone pollution in the Netherlands. Eur. Rev. Agric. Econ. 27: 75-90.

Kull, O.; Tulva, I.; Vapaavuori, E. (2003) Influence of elevated CO_2 and O_3 on *Betula pendula* Roth crown structure. Ann. Bot. 91: 559-569.

Kurczyńska, E. U.; Dmuchowski, W.; Wloch, W.; Bytnerowicz.A. (1997) The influence of air pollutants on needles and stems of scots pine (*Pinus sylvestris* L) trees. Environ. Pollut. 98: 325-334.
Kuźniak, E. (2002) Transgenic plants: an insight into oxidative stress tolerance mechanisms. Acta Physiol. Plant. (Acta Physiologiae Plantarum) 24: 97-113.
Kytöviita, M. M.; Pelloux, J.; Fontaine, V.; Botton, B.; Dizengremel, P. (1999) Elevated CO_2 does not ameliorate effects of ozone on carbon allocation in *Pinus halapensis* and *Betula pendula* in symbiosis with *Paxillus involutus*. Physiol. Plant. 106: 370-377.
Kytöviita, M. M.; Le Thiec, D.; Dizengremel, P. (2001) Elevated CO_2 and ozone reduce nitrogen acquisition by *Pinus halepensis* from its mycorrhizal symbiont. Physiol. Plant. 111: 305-312.
Lagrimini, L. M.; Rothstein, S. (1987) Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. Plant Physiol. 84: 438-442.
Laisk, A.; Kull, O.; Moldau, H. (1989) Ozone concentration in leaf intercellular air spaces is close to zero. Plant Physiol. 90: 1163-1167.
Lamb, C.; Dixon, R. A. (1997) The oxidative burst in plant disease resistance. Ann. Rev. Plant Physiol. Mol. Biol. 48: 251-275.
Landolt, W.; Günthardt-Goerg, M. S.; Pfenninger, I.; Einig, W.; Hampp, R.; Maurer, S.; Matyssek, R. (1997) Effect of fertilization on ozone-induced changes in the metabolism of birch (*Betula pendula*) leaves. New Phytol. 137: 389-397.
Landolt, W.; Bühlmann, U.; Bleuler, P.; Bucher, J. B. (2000) Ozone exposure-response relationships for biomass and root/shoot ratio of beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*), Norway spruce (*Picea abies*) and scots pine (*Pinus sylvestris*). Environ. Pollut. 109: 473-478.
Lange, O. L.; Heber, U.; Schulze, E. D.; Ziegler, H. (1989) 3-D atmospheric pollutants and plant metabolism. In: Schulze, E. D.; Lange, O. L.; Oren, R., eds. Forest decline and air pollution: a study of spruce (*Picea abies*) on acid soils. a study of spruce (*Picea abies*) on acid soils. Berlin, Federal Republic of Germany: Springer-Verlag; pp. 238-273.
Langebartels, C.; Kerner, K.; Leonardi, S.; Schraudner, M.; Trost, M.; Heller, W.; Sandermann, H., Jr. (1991) Biochemical plant responses to ozone: I differential induction of polyamine and ethylene biosynthesis in tobacco. Plant Physiol. 95: 882-889.
Langebartels, C.; Ernst, D.; Heller, W.; Lutz, C.; Payer, H. D.; Sandermann, H., Jr. (1997) Ozone responses of trees: results from controlled chamber exposures at the GSF phytotron. In: Sandermann, H.; Wellburn, A. R.; Heath, R. L., eds. Forest decline and ozone. New York, NY: Springer-Verlag; pp. 163-200 (Ecological studies: v. 127).
Larson, J. L.; Zak, D. R.; Sinsabaugh, R. L. (2002) Extracellular enzyme activity beneath temperate trees growing under elevated carbon dioxide and ozone. Soil Sci. Soc. Am. J. 66: 1848-1856.
Laurence, J. A.; Andersen, C. P. (2003) Ozone and natural systems: understanding exposure, response, and risk. Environ. Int. 29: 155-160.
Laurence, J. A.; Kohut, R. J.; Amundson, R. G.; Weinstein, D. A.; MacLean, D. C. (1996) Response of sugar maple to multiple year exposure to ozone and simulated acidic precipitation. Environ. Pollut. 92: 119-126.
Laurence, J. A.; Amundson, R. G.; Kohut, R. J.; Weinstein, D. A. (1997) Growth and water use of red spruce (*Picea rubens* Sarg.) exposed to ozone and simulated acidic precipitation for four growing seasons. For. Sci. (Bethesda, Md.) 43: 355-361.
Laurence, J. A.; Ollinger, S. V.; Woodbury, P. B. (2000) Regional impacts of ozone on forest productivity. In: Mickler, R. A.; Birdsey, R. A.; Hom, J., eds. Responses of northern U. Springer-Verlag; pp. 425-453.
Laurence, J. A.; Retzlaff, W. A.; Kern, J. S.; Lee, E. H.; Hogsett, W. E.; Weinstein, D. A. (2001) Predicting the regional impact of ozone and precipitation on the growth of loblolly pine and yellow poplar using linked TREGRO and ZELG models. For. Ecol. Manage. 146: 247-263.
Laurence, J. A.; Retzlaff, W. A.; Kern, J. S.; Lee, E. H.; Hogsett, W. E.; Weinstein, D. A. (2003) Corrigendum to "Predicting the regional impact of ozone and precipitation on the growth of loblolly pine and yellow-poplar using linked TREGRO and ZELIG models" [Forest Ecol Manage 146 (2001) 247-263]. For. Ecol. Manage. 174: 607.
Lawlor, D. W. (1998) Plant responses to global change: temperature and drought stress. In: De Kok, L. J.; Stulen, I., eds. Responses of plant metabolism to air pollution and global change. Backhuys Publishers; pp. 193-207.
Lawlor, D. W.; Keys, A. J. (1993) Understanding photosynthetic adaptation to changing climate. In: Fowden, L.; Mansfield, T.; Stoddart, J., eds. Plant adaptation to environmental stress; London, United Kingdom: Chapman and Hall; pp. Chapman and Hall; pp. 85-106.; pp. UnitedKingdom-106.

Lawson, T.; Craigon, J.; Black, C. R.; Colls, J. J.; Tulloch, A. M.; Landon, G. (2001a) Effects of elevated carbon dioxide and ozone on the growth and yield of potatoes (*Solanum tuberosum*) grown in open-top chambers. Environ. Pollut. 111: 479-491.

Lawson, T.; Craigon, J.; Tulloch, A. M.; Black, C. R.; Colls, J. J.; Landon, G. (2001b) Photosynthetic responses to elevated CO_2 and O_3 in field-grown potato (*Solanum tuberosum*). J. Plant Physiol. 158: 309-323.

Lawson, T.; Craigon, J.; Black, C. R.; Colls, J. J.; Landon, G.; Weyers, J. D. B. (2002) Impact of elevated CO_2 and O_3 on gas exchange parameters and epidermal characteristics in potato (*Solanum tuberosum* L). J. Exp. Bot. 53: 737-746.

Le Thiec, D.; Manninen, S. (2003) Ozone and water deficit reduced growth of Aleppo pine seedlings. Plant Physiol. Biochem. 41: 55-63.

Lee, E. H.; Bennett, J. H. (1982) Superoxide dismutase: a possible protective enzyme against ozone injury in snap beans (*Phaseolus vulgaris* L). Plant Physiol. 69: 1444-1449.

Lee, E. H.; Hogsett, W. E. (1999) Role of concentrations and time of day in developing ozone exposure indices for a secondary standard. J. Air Waste Manage. Assoc. 49: 669-681.

Lee, J. J.; Lewis, R. A.; Body, D. E. (1975) A field experimental system for the evaluation of the bioenvironmental effects of sulfur dioxide. In: Proceedings of the Fort Coal Field Symposium, Vol. 5; Billings, Montana: Montana Academy of Science; pp. 608-620.

Lee, E. H.; Tingey, D. T.; Hogsett, W. E. (1987) Selection of the best exposure-response model using various 7-hour ozone exposure statistics. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards.

Lee, E. H.; Tingey, D. T.; Hogsett, W. E. (1988) Evaluation of ozone exposure indices in exposure-response modeling. Environ. Pollut. 53: 43-62.

Lee, E. H.; Tingey, D. T.; Hogsett, W. E. (1989) Interrelation of experimental exposure and ambient air quality data for comparison of ozone exposure indices and estimating agricultural losses. Corvallis, OR: U.S. Environmental Protection Agency, Environmental Research Laboratory; EPA report no. EPA-600/3-89-047. Available from: NTIS, Springfield, VA; PB89-195036.

Lee, E. H.; Hogsett, W. E.; Tingey, D. T. (1994a) Alternative attainment criteria for a secondary federal standard for ozone. In: Nriagu, J. O.; Simmons, M. S.; Nriagu, J. O., eds. Environmental oxidants. New York, NY: John Wiley & Sons; pp. 549-584. (Nriagu, J. O., ed. Advances in environmental sciences and technology series: v. 28).

Lee, E. H.; Hogsett, W. E.; Tingey, D. T. (1994b) Attainment and effects issues regarding alternative secondary ozone air quality standards. J. Environ. Qual. 23: 1129-1140.

Lee, J. C.; Skelly, J. M.; Steiner, K. C.; Zhang, J. W.; Savage, J. E. (1999) Foliar response of black cherry (*Prunus serotina*) clones to ambient ozone exposure in central Pennsylvania. Environ. Pollut. 105: 325-331.

Lee, J. C.; Steiner, K. C.; Zhang, J. W.; Skelly, J. M. (2002) Heritability of ozone sensitivity in open-pollinated families of black cherry (*Prunus serotina* Ehrh.). For. Sci. 48: 111-117.

Lee, E. H.; Tingey, D. T.; Hogsett, W. E.; Laurence, J. A. (2003) History of tropospheric ozone for the San Bernardino Mountains of southern California, 1963-1999. Atmos. Environ. 37: 2705-2717.

Lefohn, A. S.; Benedict, H. M. (1982) Development of a mathematical index that describes ozone concentration, frequency and duration. Atmos. Environ. 16: 2529-2532.

Lefohn, A. S.; Foley, J. K. (1992) NCLAN results and their application to the standard-setting process: protecting vegetation from surface ozone exposures. J. Air Waste Manage. Assoc. 42: 1046-1052.

Lefohn, A. S.; Runeckles, V. C. (1987) Establishing standards to protect vegetation - ozone exposure/dose considerations. Atmos. Environ. 21: 561-568.

Lefohn, A. S.; Shadwick, D. S. (2000) Differences in trending estimates in the United States using several ozone metrics. In: Proceedings of the 93rd annual meeting of the Air & Waste Management Association; June; Salt Lake City, UT; Pittsburgh, PA: Air & Waste Management Association.

Lefohn, A. S.; Hogsett, W. E.; Tingey, D. T. (1986) A method for developing ozone exposures that mimic ambient conditions in agricultural areas. Atmos. Environ. 20: 361-366.

Lefohn, A. S.; Davis, C. E.; Jones, C. K.; Tingey, D. T.; Hogsett, W. E. (1987) Co-occurrence patterns of gaseous air pollutant pairs at different minimum concentrations in the United States. Atmos. Environ. 21: 2435-2444.

Lefohn, A. S.; Laurence, J. A.; Kohut, R. J. (1988) A comparison of indices that describe the relationship between exposure to ozone and reduction in the yield of agricultural crops. Atmos. Environ. 22: 1229-1240.

Lefohn, A. S.; Jackson, W.; Shadwick, D. S.; Knudsen, H. P. (1997) Effect of surface ozone exposures on vegetation grown in the southern Appalachian Mountains: identification of possible areas of concern. Atmos. Environ. 31: 1695-1708.

Lefohn, A. S.; Oltmans, S. J.; Dann, T.; Singh, H. B. (2001) Present-day variability of background ozone in the lower troposphere. J. Geophys. Res. [Atmos.] 106: 9945-9958.
Legge, A. H.; Grünhage, L.; Nosal, M.; Jäger, H. J.; Krupa, S. V. (1995) Ambient ozone and adverse crop response: an evaluation of North American and European data as they relate to exposure indices and critical levels. Angew. Bot. 69: 192-205.
Leitao, L.; Goulas, P.; Biolley, J. P. (2003) Time-course of Rubisco oxidation in beans (*Phaseolus vulgaris* L) subjected to a long-term ozone stress. Plant Sci. 165: 613-620.
León, J.; Lawton, M. A.; Raskin, I. (1995) Hydrogen peroxide synthesis stimulates salicylic acid biosynthesis in tobacco. Plant Physiol. 108: 1673-1683.
Lesser, V. M.; Rawlings, J. O.; Spruill, S. E.; Somerville, M. C. (1990) Ozone effects on agricultural crops: statistical methodologies and estimated dose-response relationships. Crop Sci. 30: 148-155.
Levitt, J. (1972) Responses of plants to environmental stress. New York, NY: Academic Press.
Lichtenthaler, H. K. (1977) Regulation of prenylquinone synthesis in higher plants. In: Tevini, M.; Lichtenthaler, H. K.; Davies, B. H., eds. Lipids and lipid polymers in higher plants. New York, NY: Springer-Verlag; pp. 231-258.
Lindroth, R. L.; Reich, P. B.; Tjoelker, M. G.; Volin, J. C.; Oleksyn, J. (1993) Light environment alters response to ozone stress in seedlings of *Acer saccharum* March, and hybrid *Populus* L. III. Consequences for performance of gypsy moth. New Phytol. 124: 647-654.
Lindroth, R. L.; Kopper, B. J.; Parsons, W. F. J.; Bockheim, J. G.; Karnosky, D. F.; Hendrey, G. R.; Pregitzer, K. S.; Isebrands, J. G.; Sober, J. (2001) Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). Environ. Pollut. 115: 395-404.
Liu, L.; Eriksson, K.-E. L.; Dean, J. F. D. (1995) Localization of hydrogen peroxide production in *Pisum sativum* L using epi-polarization microscopy to follow cerium perhydroxide disposition. Plant Physiol. 107: 501-506.
Lloyd, A. C.; Lents, J. M.; Green, C.; Nemeth, P. (1989) Air quality management in Los Angeles: perspectives on past and future emission control strategies. JAPCA 39: 696-703.
Loats, K. V.; Rebbeck, J. (1999) Interactive effects of ozone and elevated carbon dioxide on the growth and physiology of black cherry, green ash, and yellow poplar seedlings. Environ. Pollut. 106: 237-248.
Long, S. P. (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? Plant Cell Environ. 14: 729-739.
Lorenzini, G.; Nali, C. (1995) Analysis of vertical ozone and nitrogen oxides profiles in a *Prunus cerasifera* canopy. Int. J. Biometeorol. 39: 1-4.
Lorenzini, G.; Medeghini Bonatti, P.; Nali, C.; Baroni Fornasiero, R. (1994) The protective effect of rust infection against ozone, sulphur dioxide and paraquat toxicity symptoms in broad bean. Physiol. Mol. Plant Pathol. 45: 263-279.
Lucas, P. W.; Cottam, D. A.; Mansfield, T. A. (1987) A large-scale fumigation system for investigating interactions between air pollution and cold stress on plants. Environ. Pollut. 43: 15-28.
Lucas, P. W.; Cottam, D. A.; Sheppard, L. J.; Francis, B. J. (1988) Growth responses and delayed winter hardening in Sitka spruce following summer exposure to ozone. New Phytol. 108: 495-504.
Luo, Y. (2001) Transient ecosystem response to free-air CO_2 enrichment (FACE): experimental evidence and methods of analysis. New Phytol. 152: 3-8.
Luo, Y.; Reynolds, J. F. (1999) Validity of extrapolating field CO_2 experiments to predict carbon sequestration in natural ecosystems. Ecology 80: 1568-1583.
Luo, Y.; Canadell, J.; Mooney, H. A. (1999) Interactive effects of carbon dioxide and environmental stress on plants and ecosystems: a synthesis. In: Luo, Y.; Mooney, H. A., eds. Carbon dioxide and environmental stress. San Diego, CA: Academic Press; pp. 393-408.
Lütz, C.; Anegg, S.; Gerant, D.; Alaoi-Sossé, B.; Gérard, J.; Dizengremel, P. (2000) Beech trees exposed to high CO_2 and to simulated summer ozone levels: effects on photosynthesis, chloroplast components and leaf enzyme activity. Physiol. Plant. 109: 252-259.
Luwe, M. W. F.; Takahama, U.; Heber, U. (1993) Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia oleracea* L) leaves. Plant Physiol. 101: 969-976.
Luxmoore, R. J. (1988) Assessing the mechanisms of crop loss from air pollutants with process models. In: Heck, W. W.; Taylor, O. C.; Tingey, D. T., eds. Assessment of crop loss from air pollutants: proceedings of an international conference; October 1987; Raleigh, NC. Essex, United Kingdom: Elsevier Science Publishers, Ltd.; pp. 417-444.

Lyons, T. M.; Barnes, J. D. (1998) Influence of plant age on ozone resistance in *Plantago major*. New Phytol. 138: 83-89.
Lyons, T. M.; Barnes, J. D.; Davison, A. W. (1997) Relationships between ozone resistance and climate in European populations of *Plantago major*. New Phytol. 136: 503-510.
MacLean, D. C. (1990) Joint action of ozone and hydrogen fluoride on foliar senescence in maize. Environ. Pollut. 63: 283-292.
Madamanchi, N. R.; Anderson, J. V.; Alscher, R. G.; Cramer, C. L.; Hess, J. L. (1992) Purification of multiple forms of glutathione reductase from pea (*Pisum sativum* L) seedlings and enzyme levels in ozone-fumigated pea leaves. Plant Physiol. 100: 138-145.
Magan, N.; Kirkwood, I. A.; McLeod, A. R.; Smith, M. K. (1995) Effect of open-air fumigation with sulphur dioxide and ozone on phyllosphere and endophytic fungi of conifer needles. Plant Cell Environ. 18: 291-302.
Maggs, R.; Ashmore, M. R. (1998) Growth and yield responses of Pakistan rice (*Oryza sativa* L) cultivars to O_3 and NO_2. Environ. Pollut. 103: 159-170.
Mahoney, M. J.; Chevone, B. I.; Skelly, J. M.; Moore, L. D. (1985) Influence of mycorrhizae on the growth of loblolly pine seedlings exposed to ozone and sulfur dioxide. Phytopathology 75: 679-682.
Maier-Maercker, U. (1997) Experiments on the water balance of individual attached twigs of *Picea abies* (L.) Karst. in pure and ozone-enriched air. Trees 11: 229-239.
Maier-Maercker, U. (1998) Predisposition of trees to drought stress by ozone. Tree Physiol. 19: 71-78.
Maier-Maercker, U.; Koch, W. (1992) Histological examination of spruce needles from a long-term gas exchange experiment in pure and polluted air in the field. Trees (Berlin) 6: 186-194.
Manderscheid, R.; Jäger, H.-J.; Kress, L. W. (1992) Effects of ozone on foliar nitrogen metabolism of *Pinus taeda* L. and implications for carbohydrate metabolism. New Phytol. 121: 623-633.
Mandl, R. H.; Weinstein, L. H.; McCune, D. C.; Keveny, M. (1973) A cylindrical, open-top chamber for the exposure of plants to air pollutants in the field. J. Environ. Qual. 2: 371-376.
Mandl, R. H.; Laurence, J. A.; Kohut, R. J. (1989) Development and testing of open-top chambers for exposing large, perennial plants to air pollutants. J. Environ. Qual. 18: 534-540.
Maňkovská, B.; Godzik, B.; Badea, O.; Shparyk, Y.; Moravčík, P. (2004) Chemical and morphological characteristics of key tree species of the Carpathian Mountains. Environ. Pollut. 130: 41-54.
Manninen, A. M.; Holopainen, T.; Lyytikäinen-Saarenmaa, P.; Holopainen, J. K. (2000) The role of low-level ozone exposure and mycorrhizas in chemical quality and insect herbivore performance on Scots pine seedlings. Global Change Biol. 6: 111-121.
Manninen, S.; Siivonen, N.; Timonen, U.; Huttunen, S. (2003) Differences in ozone response between two Finnish wild strawberry populations. Environ. Exp. Bot. 49: 29-39.
Manning, W. J. (2000) Use of protective chemicals to assess the effects of ambient ozone on plants. In: Agrawal, S. B.; Agrawal, M., eds. Environmental Pollution and Plant Responses. Boca Raton, Florida: Lewis Publishers; pp. 247-258.
Manning, W. J. (2003) Detecting plant effects is necessary to give biological significance to ambient ozone monitoring data and predictive ozone standards. Environ. Pollut. 126: 375-379.
Manning, W. J.; Feder, W. A.; Papia, P. M.; Perkins, I. (1971) Influence of foliar ozone injury on root development and root surface fungi of pinto bean plants. Environ. Pollut. 1: 305-312.
Manning, W. J.; Keane, K. D. (1988) Effects of air pollutants on interactions between plants, insects, and pathogens. In: Heck, W. W.; Taylor, O. C.; Tingey, D. T., eds. Assessment of crop loss from air pollutants: proceedings of an international conference; October 1987; Raleigh, NC. Essex, United Kingdom: Elsevier Science Publishers, Ltd.; pp. 365-386.
Manning, W. J.; Krupa, S. V. (1992) Experimental methodology for studying the effects of ozone on crops and trees. In: Lefohn, A. S., ed. Surface level ozone exposures and their effects on vegetation. Chelsea, MI: Lewis Publishers, Inc.; pp. 93-156.
Manning, W. J.; Feder, W. A.; Vardaro, P. M. (1974) Suppression of oxidant injury by benomyl: effects on yields of bean cultivars in the field. J. Environ. Qual. 3: 1-3.
Manning, W. J.; Godzik, B.; Musselman, R. (2002) Potential bioindicator plant species for ambient ozone in forested mountain areas of central Europe. Environ. Pollut. 119: 283-290.
Manning, W. J.; Flagler, R. B.; Frenkel, M. A. (2003) Assessing plant response to ambient ozone: growth of ozone-sensitive loblolly pine seedlings treated with ethylenediurea or sodium erythorbate. Environ. Pollut. 126: 73-81.
Marshall, J. D.; Waring, R. H. (1985) Predicting fine root production and turnover by monitoring root starch and soil temperature. Can. J. For. Res. 15: 791-800.

Martin, M. J.; Host, G. E.; Lenz, K. E.; Isebrands, J. G. (2001) Simulating the growth response of aspen to elevated ozone: a mechanistic approach to scaling a leaf-level model of ozone effects on photosynthesis to a complex canopy architecture. Environ. Pollut. 115: 425-436.
Massman, W. J. (1993) Partitioning ozone fluxes to sparse grass and soils and the inferred resistances to dry deposition. Atmos. Environ. Part A 27A: 167-174.
Massman, W. J. (2004) Toward an ozone standard to protect vegetation based on effective dose: a review of deposition resistance and a possible metric. Atmos. Environ. In press.
Massman, W. J.; Grantz, D. A. (1995) Estimating canopy conductance to ozone uptake from observations of evapotranspiration at the canopy scale and at the leaf scale. Global Change Biol. 1: 183-198.
Massman, W. J.; Musselman, R. C.; Lefohn, A. S. (2000) A conceptual ozone dose-response model to develop a standard to protect vegetation. Atmos. Environ. 34: 745-759.
Matsumura, H. (2001) Impacts of ambient ozone and/or acid mist on the growth of 14 tree species: an open-top chamber study conducted in Japan. Water Air Soil Pollut. 130: 959-964.
Matsuyama, T.; Tamaoki, M.; Nakajima, N.; Aono, M.; Kubo, A.; Moriya, S.; Ichihara, T.; Suzuki, O. S. (2002) cDNA microarray assessment for ozone-stressed *Arabidopsis thaliana*. Environ. Pollut. 117: 191-194.
Matters, G. L.; Scandalios, J. G. (1987) Synthesis of isozymes of superoxide dismutase in maize leaves in response to O_3, SO_2, and elevated O_2. J. Exp. Bot. 38: 842-852.
Matyssek, R.; Günthardt-Goerg, M. S.; Landolt, W.; Keller, T. (1993) Whole-plant growth and leaf formation in ozonated hybrid poplar (*Populus* x *euramericana*). Environ. Pollut. 81: 207-212.
Matyssek, R.; Günthardt-Goerg, M. S.; Maurer, S.; Keller, T. (1995) Nighttime exposure to ozone reduces whole-plant production in *Betula pendula*. Tree Physiol. 15: 159-165.
Matyssek, R.; Günthardt-Goerg, M. S.; Maurer, S.; Christ, R. (2002) Tissue structure and respiration of stems of *Betula pendula* under contrasting ozone exposure and nutrition. Trees 16: 375-385.
Matyssek, R.; Wieser, G.; Nunn, A. J.; Kozovits, A. R.; Reiter, I. M.; Heerdt, C.; Winkler, J. B.; Baumgarten, M.; Häberle, K. H.; Grams, T. E. E.; Werner, H.; Fabian, P.; Havranek, W. M. (2004) Comparison between AOT40 and ozone uptake in forest trees of different species, age and site conditions. Atmos. Environ. 38: 2271-2281.
Maurer, S.; Matyssek, R. (1997) Nutrition and the ozone sensitivity of birch (*Betula pendula*) II Carbon balance, water-use efficiency and nutritional status of the whole plant. Trees 12: 11-20.
Mazarura, U. (1997) Effects of fluctuating levels of ozone and nitrogen dioxide alone and in sequence on plant growth and photosynthesis [dissertation]. University of British Columbia; Vancouver, British Columbia, Canada; 165 pp.
McAinsh, M. R.; Evans, N. G.; Montgomery, L. T. (2002) Calcium signalling in stomatal responses to pollutants. New Phytol. 153: 441-447.
McBride, J. R.; Laven, R. D. (1976) Scars as an indicator of fire frequency in the San Bernardino Mountains, California. J. For. 74: 439-442.
McBride, J. R.; Laven, R. D. (1999) Impact of oxidant air pollutants on forest succession in the mixed conifer forests of the San Bernardino Mountains. In: Miller, P. R.; McBride, J. E., eds. Oxidant air pollution impacts in the montane forests of southern California: a case study of the San Bernardino Mountains. New York, NY: Springer; pp. 338-352. (Ecological Studies, v. 134).
McCarl, B. A.; Alig, R.; Adams, D. M. (1998) Impacts of incorporating land exchanges between forestry and agriculture in sector models. J. Agric. Appl. Econ. 30: 389-401.
McCool, P. M.; Menge, J. A. (1983) Influence of ozone on carbon partitioning in tomato: potential role of carbon flow in regulation of the mycorrhizal symbiosis under conditions of stress. New Phytol. 94: 241-247.
McCool, P. M.; Menge, J. A.; Taylor, O. C. (1982) Effect of ozone injury and light stress on response of tomato to infection by the vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatus*. J. Am. Soc. Hortic. Sci. 107: 839-842.
McCool, P. M.; Musselman, R. C.; Younglove, T.; Teso, R. R. (1988) Response of kidney bean to sequential ozone exposures. Environ. Exp. Bot. 28: 307-313.
McCrady, J. K.; Andersen, C. P. (2000) The effect of ozone on below-ground carbon allocation in wheat. Environ. Pollut. 107: 465-472.
McDonald, E. P.; Kruger, E. L.; Riemenschneider, D. E.; Isebrands, J. G. (2002) Competitive status influences tree-growth responses to elevated CO_2 and O_3 in aggrading aspen stands. Funct. Ecol. 16: 792-801.
McGartland, A. M. (1987) The implications of ambient ozone standards for US agriculture: a comment and some further evidence. J. Environ. Manage. 24: 139-146.

McKee, I. F.; Farage, P. K.; Long, S. P. (1995) The interactive effects of elevated CO_2 and O_3 concentration on photosynthesis in spring wheat. Photosyn. Res. 45: 111-119.
McKee, I. F.; Bullimore, J. F.; Long, S. P. (1997a) Will elevated CO_2 concentrations protect the yield of wheat from O_3 damage? Plant Cell Environ. 20: 77-84.
McKee, I. F.; Eiblmeier, M.; Polle, A. (1997b) Enhanced ozone-tolerance in wheat grown at an elevated CO_2 concentration: ozone exclusion and detoxification. New Phytol. 137: 275-284.
McKee, I. F.; Mulholland, B. J.; Craigon, J.; Black, C. R.; Long, S. P. (2000) Elevated concentrations of atmospheric CO_2 protect against and compensate for O_3 damage to photosynthetic tissues of field-grown wheat. New Phytol. 146: 427-435.
McLaughlin, S. B.; Downing, D. J. (1995) Interactive effects of ambient ozone and climate measured on growth of mature forest trees. Nature (London) 374: 252-254.
McLaughlin, S. B.; Downing, D. J. (1996) Interactive effects of ambient ozone and climate measured on growth of mature loblolly pine trees. Can. J. For. Res. 670-681.
McLaughlin, S. B.; McConathy. (1983) Effects of SO_2 and O_3 on alocation of ^{14}C-labeled photosynthate in *Phaseolus vulgaris*. Plant Physiol. 73: 630-635.
McLaughlin, S.; Percy, K. (1999) Forest health in North America: some perspectives on actual and potential roles of climate and air pollution. Water Air Soil Pollut. 116: 151-197.
McLaughlin, S. B.; McConathy, R. K.; Duvick, D.; Mann, L. K. (1982) Effects of chronic air pollution stress on photosynthesis, carbon allocation, and growth of white pine trees. For. Sci. 28: 60-70.
McLaughlin, S. B.; Layton, P. A.; Adams, M. B.; Edwards, N. T.; Hanson, P. J.; O'Neill, E. G.; Roy, W. K. (1994) Growth-responses of 53 open-pollinated loblolly-pine families to ozone and acid-rain. J. Environ. Qual. 23: 247-257.
McLaughlin, S. B.; Shortle, W. C.; Smith, K. T. (2002) Dendroecological applications in air pollution and environmental chemistry: research needs. Dendrochronologia 20: 133-157.
McLaughlin, S. B.; Wullschleger, S. D.; Nosal, M. (2003) Diurnal and seasonal changes in stem increment and water use by yellow poplar trees in response to environmental stress. Tree Physiol. 23: 1125-1136.
McLeod, A. R.; Fackrell, J. E.; Alexander, K. (1985) Open-air fumigation of field crops: criteria and design for a new experimental system. Atmos. Environ. 19: 1639-1649.
McLeod, A. R.; Shaw, P. J. A.; Holland, M. R. (1992) The Liphook Forest Fumigation Project: studies of sulphur dioxide and ozone effects on coniferous trees. For. Ecol. Manage. 51: 121-127.
Mearns, L. O.; Rosenzweig, C.; Goldberg, R. (1997) Mean and variance change in climate scenarios: methods, agricultural applications and measures of uncertainty. Clim. Change 35: 367-396.
Meentemeyer, V. (1978) Macroclimate and lignin control of litter decomposition rates. Ecology 59: 465-475.
Mehdy, M. C. (1994) Active oxygen species in plant defense against pathogens. Plant Physiol. 105: 467-472.
Mehlhorn, H.; O'Shea, J. M.; Wellburn, A. R. (1991) Atmospheric ozone interacts with stress ethylene formation by plants to cause visible plant injury. J. Exp. Bot. 42: 17-24.
Mehlhorn, H.; Wellburn, A. R. (1987) Stress ethylene formation determines plant sensitivity to ozone. Nature (London) 327: 417-418.
Mehlhorn, H.; Wenzel, A. A. (1996) Manganese deficiency enhances ozone toxicity in bush beans (*Phaseolus vulgaris* L cv Saxa). J. Plant Physiol. 148: 155-159.
Mehlhorn, H.; Tabner, B. J.; Wellburn, A. R. (1990) Electron spin resonance evidence for the formation of free radicals in plants exposed to ozone. Physiol. Plant. 79: 377-383.
Meier, S.; Grand, L. F.; Schoeneberger, M. M.; Reinert, R. A.; Bruck, R. I. (1990) Growth, ectomycorrhizae and nonstructural carbohydrates of loblolly pine seedlings exposed to ozone and soil water deficit. Environ. Pollut. 64: 11-27.
Meinhard, M.; Rodriguez, P. L.; Grill, E. (2002) The sensitivity of ABI2 to hydrogen peroxide links the abscisic acid-response regulator to redox signalling. Planta 214: 775-782.
Mendelsohn, R.; Nordhaus, W.; Shaw, D. (1994) The impact of global warming on agriculture: a Ricardian analysis. Am. Econ. Rev. 84: 753-771.
Miglietta, F.; Peressotti, A.; Vaccari, F. P.; Zaldei, A.; Deangelis, P.; Scarascia-Mugnozza, G. (2001) Free-air CO_2 enrichment (face) of a poplar plantation: the popface fumigation system. New Phytol. 150: 465-476.
Mikkelsen, T. N.; Ro-Poulsen, H.; Hovmand, M. F.; Jensen, N. O.; Pilegard, K.; Egelov, A. H. (2004) Five-year measurements of ozone fluxes to a Danish Norway spruce canopy. Atmos. Environ. 38: 2361-2371.
Miller, P. L. (1973) Oxidant-induced community change in a mixed conifer forest. In: Naegele, J. A. , ed. Air pollution damage to vegetation. Washington, DC: American Chemical Society; pp. 101-117. (Advances in chemistry series: v. 122).

Miller, P. R.; Elderman, M. J. (1977) Photochemical oxidant air pollutant effects on a mixed conifer forest ecosystem: a progress report, 1976. Corvallis, OR: U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory; EPA report no. EPA-600/3-77-104. Available from: NTIS, Springfield, VA; PB-274531.

Miller, P. R.; McBride, J. R. (1999a) Oxidant air pollution impacts in the Montane forests of southern California: a case study of the San Bernardino Mountains. (Ecological Studies, v. 134).

Miller, P. R.; McBride, J. R. (1999b) Assessment of ecological risks and implications for policy and management in the San Bernardino Mountains. In: Miller, P. R.; McBride, J. R., eds. Oxidant air pollution impacts in the Montane forests of southern California: a case study of the San Bernardino Mountains. New York, NY: Springer; pp. 397-404. (Ecological Studies, v. 134).

Miller, P. R.; Millecan, A. A. (1971) Extent of oxidant air pollution damage to some pines and other conifers in California. Plant Dis. Rep. 55: 555-559.

Miller, J. E.; Pursley, W. A.; Heagle, A. S. (1994) Effects of ethylenediurea on snap bean at a range of ozone concentrations. J. Environ. Qual. 23: 1082-1089.

Miller, P. R.; Rechel, J. (1999) Temporal changes in crown condition indices, needle litterfall, and collateral needle injuries of Ponderosa and Jeffrey pines. In: Miller, P. R.; McBride, J. R., eds. Oxidant air pollution impacts in the Montane forests of southern California: a case study of the San Bernardino Mountains. New York, NY: Springer; pp. 164-178.

Miller, P. R.; Parmeter, J. R., Jr.; Taylor, O. C.; Cardiff, E. A. (1963) Ozone injury to the foliage of *Pinus ponderosa*. Phytopathology 53: 1072-1076.

Miller, P. R.; Parmeter, J. R., Jr.; Flick, B. H.; Martinez, C. W. (1969) Ozone dosage response of ponderosa pine seedlings. J. Air Pollut. Control Assoc. 19: 435-438.

Miller, P. R.; McCutchan, M. H.; Milligan, H. P. (1972) Oxidant air pollution in the Central Valley, Sierra Nevada foothills, and Mineral King Valley of California. Atmos. Environ. 6: 623-633.

Miller, P. R.; Taylor, O. C.; Wilhour, R. G. (1982) Oxidant air pollution effects on a western coniferous forest ecosystem. Corvallis, OR: U.S. Environmental Protection Agency, Environmental Research Laboratory; EPA report no. EPA-600/D-82-276. Available from: NTIS, Springfield, VA; PB83-189308.

Miller, P. R.; McBride, J. R.; Schilling, S. L.; Gomez, A. P. (1989) Trend of ozone damage to conifer forests between 1974 and 1988 in the San Bernardino Mountains of southern California. In: Olson, R. K.; Lefohn, A. S., eds. Effects of air pollution on western forests [an A&WMA symposium; June; Anaheim, CA]. Air and Waste Management Association; pp. 309-323. (APCA transactions series, no. 16).

Miller, J. E.; Booker, F. L.; Fiscus, E. L.; Heagle, A. S.; Pursley, W. A.; Vozzo, S. F.; Heck, W. W. (1994) Ultraviolet-B radiation and ozone effects on growth, yield, and photosynthesis of soybean. J. Environ. Qual. 23: 83-91.

Miller, P. R.; De Bauer, L. I.; Quevedo, A.; Hernandez-Tejeda, T. (1994) Comparison of ozone exposures in forested regions near Mexico City and Los Angeles. Atmos. Environ. 28: 141-148.

Miller, J. E.; Heagle, A. S.; Pursley, W. A. (1998) Influence of ozone stress on soybean response to carbon dioxide enrichment: II Biomass and development. Crop Sci. 38: 122-128.

Miller, J. D.; Arteca, R. N.; Pell, E. J. (1999) Senescence-associated gene expression during ozone-induced leaf senescence in *Arabidopsis*. Plant Physiol. 120: 1015-1024.

Mills, G. (2002) Modification of plant response by environmental conditions. In: Bell, J. N. B.; Treshow, M., eds. Air pollution and plant life. 2nd ed. Chichester, United Kingdom: John Wiley & Sons Ltd.; pp. 343-358.

Mills, G.; Ball, G.; Hayes, F.; Fuhrer, J.; Skarby, L.; Gimeno, B.; De Temmerman, L.; Heagle, A.; Members of the ICP Vegetation programme. (2000) Development of a multi-factor model for predicting the effects of ambient ozone on the biomass of white clover. Environ. Pollut. 109: 533-542.

Minnich, R. A. (1999) Vegetation, fire regimes, and forest dynamics. In: Oxidant air pollution impacts in the montane forests southern California: a case study of the San Bernardino mountains. New York, NY: Springer; pp. 44-80 (Ecological studies: v. 134).

Minnich, R. A.; Padgett, P. E. (2003) Geology, climate, and vegetation of the Sierra Nevada and the mixed-conifer zone: an introduction to the ecosystem. In: Bytnerowicz, A.; Arbaugh, M. J.; Alonso, R., eds. Ozone Air Pollution in the Sierra Nevada: Distribution and Effects on Forests. New York, NY: Elsevier; pp. 1-31.

Minnich, R. A.; Barbour, M. G.; Burk, J. H.; Fernau, R. F. (1995) Sixty years of change in California conifer forests of the San Bernardino mountains. Conserv. Biol. 9: 902-914.

Mira, H.; Mártinez, N.; Peñarrubia, L. (2002) Expression of a vegetative-storage-protein gene form Arabidopsis is regulated by copper, senescence and ozone. Planta 214: 939-946.

Moeder, W.; Barry, C. S.; Tauriainen, A. A.; Betz, C.; Tuomainen, J.; Ultrainen, M.; Grierson, D.; Sandermann, H.; Langebartels, C.; Kangasjarvi, J. (2002) Ethylene synthesis regulated by biphasic induction of 1-aminocyclopropane-1-carboxylic acid synthase and 1-aminocyclopropane-1-carboxylic acid oxidase genes is required for hydrogen peroxide accumulation and cell death in ozone-exposed tomato. Plant Physiol. 130: 1918-1926.

Moldau, H. (1998) Hierarchy of ozone scavenging reactions in the plant cell wall. Physiol. Plant. 104: 617-622.

Moldau, H.; Bichele, I. (2002) Plasmalemma protection by the apoplast as assessed from above zero ozone concentration in leaf intercellular air spaces. Planta 214: 484-487.

Momen, B.; Helms, J. A. (1996) Effects of simulated acid rain and ozone on foliar chemistry of field-grown *Pinus ponderosa* seedlings and mature trees. Environ. Pollut. 91: 105-111.

Momen, B.; Helms, J. A.; Criddle, R. S. (1996) Foliar metabolic heat rate of seedlings and mature trees of *Pinus ponderosa* exposed to simulated acid rain and ozone. Plant Cell Environ. 19: 747-753.

Momen, B.; Anderson, P. D.; Helms, J. A.; Houpis, J. L. J. (1997) Acid rain and ozone effects on gas exchange of *Pinus ponderosa*: a comparison between trees and seedlings. Int. J. Plant Sci. 158: 617-621.

Momen, B.; Anderson, P. D.; Helms, J. A. (1999) Temperature dependency of acid-rain effect on photosynthesis of *Pinus ponderosa*. For. Ecol. Manage. 113: 223-230.

Momen, B.; Anderson, P. D.; Houpis, J. L. J.; Helms, J. A. (2002) Growth of ponderosa pine seedlings as affected by air pollution. Atmos. Environ. 36: 1875-1882.

Monteith, J. L.; Elston, J. (1993) Climatic constraints on crop production. In: Fowden, L.; Mansfield, T.; Stoddart, J., eds. Plant adaptation to environmental stress. London, United Kingdom: Chapman and Hall; pp. 3-18.

Morgan, P. B.; Ainsworth, E. A.; Long, S. P. (2003) How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. Plant Cell Environ. 26: 1317-1328.

Morgan, P. B.; Bernacchi, C. J.; Ort, D. R.; Long, S. P. (2004) An in vivo analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. Plant Physiol. 135: 2348-2357.

Morgan, P. B.; Mies, T. A.; Bollero, G. A.; Nelson, R. L.; Long, S. P. (2006) Season-long elevation of ozone concentration to projected 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean. New Phytol.: in press.

Morison, J. I. L.; Lawlor, D. W. (1999) Interactions between increasing CO_2 concentration and temperature on plant growth. Plant Cell Environ. 22: 659-682.

Mortensen, L. M. (1998) Growth responses of seedlings of six *Betula pubescens* Ehrh Provenances to six ozone exposure regimes. Scand. J. For. Res. 13: 189-196.

Mortensen, L. M. (1999) Foliar injuries caused by ozone in *Betula pubescens* Ehrh and *Phleum pratense* L as influenced by climatic conditions before and during O_3 exposure. Acta Agric. Scand. B 49: 44-49.

Mortensen, L.; Bastrup-Birk, A. (1996) Critical levels for O_3 for biomass production in white clover. In: Kärenlampi, L.; Skärby, L., eds. Critical levels for ozone in Europe: testing and finalizing the concepts: UN-ECE workshop report. testing and finalizing the concepts: UN-ECE workshop report. UN-ECE Convention on Long-Range Transboundary Air Pollution; pp. Kuopio,Finland-Kuopio, Finland.

Mudd, J. B. (1996) Biochemical basis for the toxicity of ozone. In: Yunus, M.; Iqbal, M., eds. Plant response to air pollution. John Wiley & Sons; pp. 267-283.

Mudd, J. B.; Kozlowski, T. T., eds. (1975) Responses of plants to air pollution. New York, NY: Academic Press. (Physiological ecology, a series of monographs, texts, and treatises).

Mudd, J. B.; Leavitt, R.; Ongun, A.; McManus, T. T. (1969) Reaction of ozone with amino acids and proteins. Atmos. Environ. 3: 669-681.

Mudd, J. B.; McManus, T. T.; Ongun, A. (1971) Inhibition of lipid metabolism in chloroplasts by ozone. In: Englund, H. M.; Beery, W. T. eds. Proceedings of the second clean air congress; New York, NY: Academic Press; pp. 256-260.

Mudd, J. B.; Dawson, P. J.; Adams, J. R.; Wingo, J.; Santrock, J. (1996) Reaction of ozone with enzymes of erythrocyte membranes. Arch. Biochem. Biophys. 335: 145-151.

Mudd, J. B.; Dawson, P. J.; Santrock, J. (1997a) Ozone does not react with human erythrocyte membrane lipids. Arch. Biochem. Biophys. 341: 251-258.

Mudd, J. B.; Dawson, P. J.; Tseng, S.; Liu, F. P. (1997b) Reaction of ozone with protein tryptophans: band III, serum albumin, and cytochrome C. Arch. Biochem. Biophys. 338: 143-149.

Mueller, S. F. (1994) Characterization of ambient ozone levels in the Great Smoky Mountains National Park. J. Appl. Meteorol. 33: 465-472.

Mukammal, E. I. (1965) Ozone as a cause of tobacco injury. Agric. Meteorol. 2: 145-165.

Mulchi, C. L.; Slaughter, L.; Saleem, M.; Lee, E. H.; Pausch, R.; Rowland, R. (1992) Growth and physiological characteristics of soybean in open-top chambers in response to ozone and increased atmospheric CO_2. Agric. Ecosyst. Environ. 38: 107-118.

Mulchi, C.; Rudorff, B.; Lee, E.; Rowland, R.; Pausch, R. (1995) Morphological responses among crop species to full-season exposures to enhanced concentrations of atmospheric CO_2 and O_3. Water Air Soil Pollut. 85: 1379-1386.

Mulholland, B.; Craigon, J.; Black, C.; Atherton, J.; Landon, G. (1997a) Effects of elevated carbon dioxide and ozone on the growth and yield of spring wheat (*Triticum aestivum* L). J. Exper. Bot. 48: 113-122.

Mulholland, B. J.; Craigon, J.; Black, C. R.; Colls, J. J.; Atherton, J.; Landon, G. (1997b) Impact of elevated atmospheric CO_2 and O_3 on gas exchange and chlorophyll content in spring wheat (*Triticum aestivum* L). J. Exp. Bot. 48: 1853-1863.

Mulholland, B. J.; Craigon, J.; Black, C. R.; Colls, J. J.; Atherton, J.; Landon, G. (1998a) Growth, light interception and yield responses of spring wheat (*Triticum aestivum* L) grown under elevated CO_2 and O_3 in open-top chambers. Global Change Biol. 4: 121-130.

Mulholland, B. J.; Craigon, J.; Black, C. R.; Colls, J. J.; Atherton, J.; Landon, G. (1998b) Effects of elevated CO_2 and O_3 on the rate and duration of grain growth and harvest index in spring wheat (*Triticum aestivum* L). Global Change Biol. 4: 627-635.

Muntifering, R. B.; Crosby, D. D.; Powell, M. C.; Chappelka, A. H. (2000) Yield and quality characteristics of bahiagrass (*Paspalum notatum*) exposed to ground-level ozone. Anim. Feed Sci. Technol. 84: 243-256.

Murphy, J. J.; Deluki, M. A.; McCubbin, D. R.; Kim, H. J. (1999) The cost of crop damage caused by ozone air pollution from motor vehicles. J. Environ. Manage. 55: 273-289.

Musselman, R. C.; Hale, B. A. (1997) Methods for controlled and field ozone exposures of forest tree species in North America. In: Sanderman, H.; Wellburn, A. R.; Heath, R. L., eds. Forest decline and ozone. Berlin, Germany: Springer Verlag; pp. 277-315 (Ecological Studies v. 127).

Musselman, R. C.; Massman, W. J. (1999) Ozone flux to vegetation and its relationship to plant response and ambient air quality standards. Atmos. Environ. 33: 65-73.

Musselman, R. C.; Minnick, T. J. (2000) Nocturnal stomatal conductance and ambient air quality standards for ozone. Atmos. Environ. 34: 719-733.

Musselman, R. C.; Oshima, R. J.; Gallavan, R. E. (1983) Significance of pollutant concentration distribution in the response of 'red kidney' beans to ozone. J. Am. Soc. Hortic. Sci. 108: 347-351.

Musselman, R. C.; Huerta, A. J.; McCool, P. M.; Oshima, R. J. (1986) Response of beans to simulated ambient and uniform ozone distributions with equal peak concentration. J. Am. Soc. Hortic. Sci. 111: 470-473.

Musselman, R. C.; McCool, P. M.; Younglove, T. (1988) Selecting ozone exposure statistics for determining crop yield loss from air pollutants. Environ. Pollut. 53: 63-78.

Musselman, R. C.; Younglove, T.; McCool, P. M. (1994) Response of *Phaseolus vulgaris* L to differing ozone regimes having indentical total exposure. Atmos. Environ. 28: 2727-2731.

Muzika, R. M.; Guyette, R. P.; Zielonka, T.; Liebhold, A. M. (2004) The influence of O_3, NO_2, and SO_2 on growth of *Picea abies* and *Fagus sylvatica* in the Carpathian Mountains. Environ. Pollut. 130: 65-71.

Nali, C.; Pucciariello, C.; Lorenzini, G. (2002) Mapping ozone critical levels for vegetation in central Italy. Water Air Soil Pollut. 141: 337-347.

Nash, T. H., III; Sigal, L. L. (1999) Epiphytic lichens in the San Bernardino mountains in relation to oxidant gradients. In: Miller, P. R.; McBride, J. R., eds. Oxidant air pollution impacts in the montane forests of southern California: a case study of the San Bernardino Mountains. New York, NY: Springer-Verlag; pp 223-234. (Ecological studies: v. 134).

Neill, S. J.; Desikan, R.; Clarke, A.; Hurst, R. D.; Hancock, J. T. (2002) Hydrogen peroxide and nitric oxide as signalling molecules in plants. J. Exper. Bot. 53: 1237-1247.

Neufeld, H. S.; Renfro, J. R.; Hacker, W. D.; Silsbee, D. (1992) Ozone in Great Smoky Mountains National Park: dynamics and effects on plants. In: Berglund, R. L. ed. Tropospheric ozone and the environment II: effects, modeling and control [papers from an international specialty conference]; November; Atlanta, GA; Pittsburgh, PA: Air & Waste Management Association; pp. 594-617. (A&WMA transactions series no. 20).

Neufeld, H. S.; Lee, E. H.; Renfro, J. R.; Hacker, W. D.; Yu, B. H. (1995) Sensitivity of seedlings of black cherry (*Prunus serotina* Ehrh) to ozone in Great Smoky Mountains National Park I Exposure-response curves for biomass. New Phytol. 130: 447-459.

Neufeld, H. S.; Lee, E. H.; Renfro, J. R.; Hacker, W. D. (2000) Seedling insensitivity to ozone for three conifer species native to Great Smoky Mountains National Park. Environ. Pollut. 108: 141-151.

Niewiadomska, E.; Gaucher-Veilleux, C.; Chevrier, N.; Mauffette, Y.; Dizengremel, P. (1999) Elevated CO_2 does not provide protection against ozone considering the activity of several antioxidant enzymes in the leaves of sugar maple. J. Plant Physiol. 155: 70-77.

Nijs, I.; Teughels, H.; Blum, H.; Hendrey, G.; Impens, I. (1996) Simulation of climate change with infrared heaters reduces the productivity of *Lolium perenne* L in summer. Environ. Exp. Bot. 36: 271-280.

Nikolov, N.; Zeller, K. (2003) Modeling coupled interactions of carbon, water, and ozone exchange between terrestrial ecosystems and the atmosphere. Environ. Pollut. 124: 231-246.

Noormets, A.; McDonald, E. P.; Dickson, R. E.; Kruger, E. L.; Sôber, A.; Isebrands, J. G.; Karnosky, D. F. (2001a) The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain in aspen. Trees 15: 262-270.

Noormets, A.; Sôber, A.; Pell, E. J.; Dickson, R. E.; Posila, G. K.; Sôber, J.; Isebrands, J. G.; Karnosky, D. F. (2001b) Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremuloides* Michx) clones exposed to elevated CO_2 and/or O_3. Plant Cell Environ. 24: 327-336.

Norby, R. J.; Weerasuriya, Y.; Hanson, P. J. (1989) Induction of nitrate reductase activity in red spruce needles by NO_2 and HNO_3 vapor. Can. J. For. Res. 19: 889-896.

Norby, R. J.; Wullschleger, S. D.; Gunderson, C. A.; Johnson, D. W.; Ceulemans, R. (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ. 22: 683-714.

Norton, B. G. (1987) Why preserve natural variety? Princeton, NJ: Princeton University Press.

Nouchi, I.; Toyama, S. (1988) Effects of ozone and peroxyacetyl nitrate on polar lipids and fatty acids in leaves of morning glory and kidney bean. Plant Physiol. 87: 638-646.

Novak, K.; Schaub.M.; Kräuchi, N.; Hug, C.; Landolt, W.; Bleuler, P. (2003) Ozone air pollution and foliar injury development on native plants of Switzerland. Environ. Pollut. 125: 41-52.

Nunn, A. J.; Reiter, I. M.; Haberle, K. H.; Werner, H.; Langebartels, C.; Sandermann, H.; Heerdt, C.; Fabian, P.; Matyssek, R. (2002) "Free-air" ozone canopy fumigation in an old-growth mixed forest: concept and observations in beech. Phyton 42: 105-119.

Nussbaum, S.; Fuhrer, J. (2000) Difference in ozone uptake in grassland species between open-top chambers and ambient air. Environ. Pollut. 109: 463-471.

Nussbaum, S.; Geissmann, M.; Fuhrer, J. (1995a) Effects of nitric oxide and ozone on spring wheat (*Triticum aestivum*). Water Air Soil Pollut. 85: 1449-1454.

Nussbaum, S.; Geissmann, M.; Fuhrer, J. (1995b) Ozone exposure-response relationships for mixtures of perennial ryegrass and white clover depend on ozone exposure patterns. Atmos. Environ. 29: 989-995.

Nussbaum, S.; Ammann, M.; Fuhrer, J. (1999) Foliar absorption and use of airborne oxidized nitrogen by terrestrial plants. In: Srivastava, H. S.; Singh, R. P., eds. Nitrogen nutrition and plant growth. New Delhi, India: Oxford & IBH Publishing Co. Pvt. Ltd; pp. 101-172.

Nussbaum, S.; Bungener, P.; Geissmann, M.; Fuhrer, J. (2000a) Plant-plant interactions and soil moisture might be important in determining ozone impacts on grasslands. New Phytol. 147: 327-335.

Nussbaum, S.; Geissman, M.; Saurer, M.; Siegwolf, R.; Fuhrer, J. (2000b) Ozone and low concentrations of nitric oxide have similar effects on carbon isotope discrimination and gas exchange in leaves of wheat (*Triticum aestivum* L). J. Plant Physiol. 156: 741-745.

Nussbaum, S.; Remund, J.; Rihm, B.; Mieglitz, K.; Gurtz, J.; Fuhrer, J. (2003) High-resolution spatial analysis of stomatal ozone uptake in arable crops and pastures. Environ. Int. 29: 385-392.

Odum, E. P. (1963) Ecology. New York, NY: Holt, Rinehart and Winston. (Modern biology series).

O'Gara, P. J. (1922) Sulfur dioxide and fume problems and their solution. In: Olsen, J. C., ed. Proceedings of the 14th semiannual meeting of the American Institute of Chemical Engineers; June; Niagara Falls, Canada. Proceedings and papers of the meeting summarized in J. Ind. Eng. Chem. 14: 744-745 by J. C. Olsen.

Ojanperä, K.; Pätsikkä, E.; Yläranta, T. (1998) Effects of low ozone exposure of spring wheat on net CO_2 uptake, Rubisco, leaf senescence and grain filling. New Phytol. 138: 451-460.

Oksanen, E. J. (2001) Increasing tropospheric ozone level reduced birch (*Betula pendula*) dry mass within a five years period. Water Air Soil Pollut. 130: 947-952.

Oksanen, E. (2003a) Responses of selected birch (*Betula pendula* Roth) clones to ozone change over time. Plant Cell Environ. 26: 875-886.

Oksanen, E. (2003b) Physiological responses of birch (*Betula pendula*) to ozone: a comparison between open-soil-grown trees exposed for six growing seasons and potted seedlings exposed for one season. Tree Physiol. 23: 603-614.

Oksanen, E.; Holopainen, T. (2001) Responses of two birch (*Betula pendula* Roth) clones to different ozone profiles with similar AOT40 exposure. Atmos. Environ. 35: 5245-5254.

Oksanen, E.; Rousi, M. (2001) Differences in Betula origins in ozone sensitivity based open-field fumigation experiment over two growing seasons. Can. J. For. Res. 31: 804-811.
Oksanen, E.; Saleem, A. (1999) Ozone exposure results in various carry-over effects and prolonged reduction in biomass in birch (*Betula pendula* Roth). Plant Cell Environ. 22: 1401-1411.
Oksanen, E.; Amores, A.; Kokko, H.; Santamaria, J. M.; Kärenlampi, L. (2001a) Genotypic variation in growth and physiological responses of Finnish hybrid aspen (*Populus tremuloides* x *P tremula*) to elevated tropospheric ozone concentration. Tree Physiol. 21: 1171-1181.
Oksanen, E.; Sober, J.; Karnosky, D. F. (2001b) Interactions of elevated CO_2 and ozone in leaf morphology of aspen (*Populus tremuloides*) and birch (Betula papyrifera) in aspen FACE experiment. Environ. Pollut. 115: 437-446.
Ollerenshaw, J. H.; Lyons, T. (1999) Impacts of ozone on the growth and yield of field-grown winter wheat. Environ. Pollut. 106: 67-72.
Ollerenshaw, J. H.; Lyons, T.; Barnes, J. D. (1999) Impacts of ozone on the growth and yield of field-grown winter oilseed rape. Environ. Pollut. 104: 53-59.
Ollinger, S. V.; Aber, J. D.; Reich, P. B. (1997) Simulating ozone effects on forest productivity: interactions among leaf-, canopy-, and stand-level processes. Ecol. Appl. 7: 1237-1251.
Ollinger, S. V.; Aber, J. D.; Federer, C. A. (1998) Estimating regional forest productivity and water yield using an ecosystem model linked to a GIS. Landscape Ecol. 13: 323-334.
Olszyk, D. M.; Bytnerowicz, A.; Kats, G.; Dawson, P. J.; Wolf, J.; Thompson, C. R. (1986a) Crop effects from air pollutants in air exclusion systems vs field chambers. J. Environ. Qual. 15: 417-422.
Olszyk, D. M.; Kats, G.; Dawson, P. J.; Bytnerowicz, A.; Wolf, J.; Thompson, C. R. (1986b) Characteristics of air exclusion systems vs. chambers for filed air pollution studies. J. Environ. Qual. 15: 326-334.
Olszyk, D. M.; Takemoto, B. K.; Kats, G.; Dawson, P. J.; Morrison, C. L.; Preston, J. W.; Thompson, C. R. (1992) Effects of open-top chambers on 'Valencia' orange trees. J. Environ. Qual. 21: 128-134.
Olszyk, D. M.; Tingey, D. T.; Watrud, L.; Seidler, R.; Andersen, C. (2000) Interactive effects of O_3 and CO_2: implications for terrestrial ecosystems. In: Singh, S. N., ed. Trace gas emissions and plants. Dordrecht, The Netherlands: Kluwer Academic Publishers; pp. 97-136.
Olszyk, D. M.; Johnson, M. G.; Phillips, D. L.; Seidler, R. J.; Tingey, D. T.; Watrud, L. S. (2001) Interactive effects of CO_2 and O_3 on a ponderosa pine/litter/soil mesocosm. Environ. Pollut. 115: 447-462.
Ommen, O. E.; Donnelly, A.; Vanhoutvin, S.; Van Oijen, M.; Manderscheid, R. (1999) Chlorophyll content of spring wheat flag leaves grown under elevated CO_2 concentrations and other environmental stresses within the 'ESPACE-wheat' project. Eur. J. Agron. 10: 197-203.
Ordin, L.; Hall, M. A.; Kindinger, J. I. (1969) Oxidant induced inhibition of enzymes involved in cell wall polysaccharide synthesis. Arch. Environ. Health 18: 623-626.
Oren, R.; Ellsworth, D. S.; Johnsen, K. H.; Phillips, N.; Ewers, B. E.; Maier, C.; Schäfer, K. V. R.; McCarthy, H.; Hendrey, G.; McNulty, S. G.; Katul, G. G. (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature (London) 411: 469-472.
Orendovici, T.; Skelly, J. M.; Ferdinand, J. A.; Savage, J. E.; Sanz, M.-J.; Smith, G. C. (2003) Response of native plants of northeastern United States and southern Spain to ozone exposures; determining exposure/response relationships. Environ. Pollut. 125: 31-40.
Ormrod, D. P.; Beckerson, D. W. (1986) Polyamines as antiozonants for tomato. HortScience 21: 1070-1071.
Ormrod, D. P.; Landry, L. G.; Conklin, P. L. (1995) Short-term UV-B radiation and ozone exposure effects on aromatic secondary metabolite accumulation and shoot growth of flavonoid-deficient *Arabidopsis* mutants. Physiol. Plant. 93: 602-610.
Örvar, B. L.; Ellis, B. E. (1997) Transgenic tobacco plants expressing antisense RNA for cytosolid ascorbate peroxidase show increased susceptibility to ozone injury. Plant J. 11: 1297-1305.
Oshima, R. J.; Poe, M. P.; Braegelmann, P. K.; Baldwin, D. W.; Van Way, V. (1976) Ozone dosage-crop loss function for alfalfa: a standardized method for assessing crop losses from air pollutants. J. Air Pollut. Control Assoc. 26: 861-865.
Oshima, R. J.; Braegelmann, P. K.; Baldwin, D. W.; Van Way, V.; Taylor, O. C. (1977) Responses of five cultivars of fresh market tomato to ozone: a contrast of cultivar screening with foliar injury and yield. J. Am. Soc. Hortic. Sci. 102: 286-289.
Ottosson, S.; Wallin, G.; Skärby, L.; Karlsson, P. E.; Medin, E. L.; Räntfors, M.; Pleijel, H.; Selldén, G. (2003) Four years of ozone exposure at high or low phosphorus reduced biomass in Norway spruce. Trees (Heidelberg, Ger.) 17: 299-307.

Overmyer, K.; Tuominen, H.; Kettunen, R.; Betz, C.; Langebartels, C.; Sandermann, H., Jr.; Kangasjärvi, J. (2000) Ozone-sensitive Arabidopsis *rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell 12: 1849-1862.

Overmyer, K.; Brosché, M.; Kangasjärvi, J. (2003) Reactive oxygen species and hormonal control of cell death. Trends Plant Sci. 8: 335-342.

Pääkkönen, E.; Holopainen, T. (1995) Influence of nitrogen supply on the response of clones of birch (*Betula pendula* Roth) to ozone. New Phytol. 129: 595-603.

Pääkkönen, E.; Holopainen, T.; Kärenlampi, L. (1996) Relationships between open-field ozone exposures and growth and senescence of birch (*Betula pendula* and *Betula pubescens*). In: Kärenlampi, L.; Skärby, L. eds. Critical levels for ozone in Europe: testing and finalizing the concepts: UN-ECE workshop report. UN-ECE Convention on Long-Range Transboundary Air Pollution; April; Kuopio, Finland. Kuopio, Finland: University of Kuopio, Department of Ecology and Environmental Science; pp. 298-302.

Pääkkönen, E.; Holopainen, T.; Kärenlampi, L. (1997) Variation in ozone sensitivity among clones of *Betula pendula* and *Betula pubescens*. Environ. Pollut. 95: 37-44.

Pääkkönen, E.; Günthardt-Georg, M. S.; Holopainen, T. (1998) Responses of leaf processes in a sensitive birch (*Betula pendula* Roth) clone to ozone combined with drought. Ann. Bot. 82: 49-59.

Paludan-Müller, G.; Saxe, H.; Leverenz, J. W. (1999) Responses to ozone in 12 provenances of European beech (*Fagus sylvatica*): genotypic variation and chamber effects on photosynthesis and dry-matter partitioning. New Phytol. 144: 261-273.

Panek, J. A. (2004) Ozone uptake, water loss and carbon exchange dynamics in annually drought-stressed *Pinus ponderosa* forests: measured trends and parameters for uptake modeling. Tree Physiol. 24: 277-290.

Panek, J. A.; Goldstein, A. H. (2001) Responses of stomatal conductance to drought in ponderosa pine: implications for carbon and ozone uptake. Tree Physiol. 21: 337-344.

Panek, J.; Kurpius, M. R.; Goldstein, A. H. (2002) An evaluation of ozone exposure metrics for a seasonally drought-stressed ponderosa pine ecosystem. Environ. Pollut. 117: 93-100.

Panek, J. A.; Baldocchi, D. D.; Goldstein, A. H. (2003) The need for spatially and functionally integrated models of ozone deposition to Sierra Nevada forests. In: Bytnerowicz, A.; Arbaugh, M. J.; Alonso, R., eds. Ozone air pollution in the Sierra Nevada: distribution and effects on forests. New York, NY: Elsevier Science Ltd; pp. 325-357. (Developments in environmental science: v. 2).

Patterson, M. T.; Rundel, P. W. (1995) Stand characteristics of ozone-stressed populations of *Pinus jeffreyi* (Pinaceae): extent, development, and physiological consequences of visible injury. Am. J. Bot. 82: 150-158.

Pauls, K. P.; Thompson, J. E. (1980) In vitro simulation of senescence-related membrane damage by ozone-induced lipid peroxidation. Nature (London) 283: 504-506.

Payne, G.; Middlesteadt, W.; Desai, N.; Williams, S.; Dincher, S.; Carnes, M.; Ryals, J. (1989) Isolation and sequence of a genomic clone encoding the basic form of pathogenesis-related protein-1 from *Nicotiana tabacum*. Plant Mol. Biol. 12: 595-596.

Payne, G.; Ahl, P.; Moyer, M.; Harper, A.; Beck, J.; Meins, F., Jr.; Ryals, J. (1990) Isolation of complementary DNA clones encoding pathogenesis-related proteins P and Q, two acidic chitinases from tobacco. Proc. Natl. Acad. Sci. U. S. A. 87: 98-102.

Pearce, D. W. (1993) Economic values and the natural world. London, United Kingdom: Earthscan Publications, Ltd..

Pearson, M.; Mansfield, T. A. (1993) Interacting effects of ozone and water stress on the stomatal resistance of beech (*Fagus sylvatica* L). New Phytol. 123: 351-358.

Pearson, M.; Mansfield, T. A. (1994) Effects of exposure to ozone and water stress on the following season's growth of beech (*Fagus sylvatica* L). New Phytol. 126: 511-515.

Pearson, S.; Davison, A. W.; Reiling, K.; Ashenden, T.; Ollerenshaw, J. H. (1996) The effects of different ozone exposures on three contrasting populations of *Plantago major*. New Phytol. 132: 493-502.

Pel, Z.-M.; Murata, Y.; Benning, G.; Thomine, S.; Klüsener, B.; Allen, G. J.; Grill, E.; Schroeder, J. I. (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature (London) 406: 731-734.

Pell, E. J.; Pearson, N. S. (1984) Ozone-induced reduction in quantity and quality of two potato cultivars. Environ. Pollut. Ser. A 35: 345-352.

Pell, E. J.; Eckardt, N.; Glick, R. E. (1994) Biochemical and molecular basis for impairment of photosynthetic potential. Photosynth. Res. 39: 453-462.

Pell, E. J.; Sinn, J. P.; Johansen, C. V. (1995) Nitrogen supply as a limiting factor determining the sensitivity of *Populus tremuloides* Michx to ozone stress. New Phytol. 130: 437-446.

Pell, E. J.; Schlagnhaufer, C. D.; Arteca, R. N. (1997) Ozone-induced oxidative stress: mechanisms of action and reaction. Physiol. Plant. 100: 264-273.
Pell, E. J.; Sinn, J. P.; Brendley, B. W.; Samuelson, L.; Vinten-Johansen, C.; Tien, M.; Skillman, J. (1999) Differential response of four tree species to ozone-induced acceleration of foliar senescence. Plant Cell Environ. 22: 779-790.
Pellinen, R.; Palva, T.; Kangasjävi, J. (1999) Subcellular localization of ozone-induced hydrogen peroxide production in birch (*Betula pendula*) leaf cells. Plant. J. 20: 349-356.
Pelloux, J.; Jolivet, Y.; Fontaine, V.; Banvoy, J.; Dizengremel, P. (2001) Changes in Rubisco and Rubisco activase gene expression and polypeptide content in *Pinus halepensis* M subjected to ozone and drought. Plant Cell Environ. 24: 123-131.
Penel, C. (1986) The role of calcium in the control of peroxidase activity. In: Greppin, H.; Penel, C.; Gasper, T., eds. Molecular and physiological aspects of plant peroxidases. Universite de Genieve, Centre de Botanique; pp. 155-164.
Percy, K. E.; Jensen, K. F.; McQuattie, C. J. (1992) Effects of ozone and acidic fog on red spruce needle epicuticular wax production, chemical composition, cuticular membrane ultrastructure and needle wettability. New Phytol. 122: 71-80.
Percy, K. E.; Awmack, C. S.; Lindroth, R. L.; Kubiske, M. E.; Kopper, B. J.; Isebrands, J. G.; Pregitzer, K. S.; Hendry, G. R.; Dickson, R. E.; Zak, D. R.; Oksanen, E.; Sober, J.; Harrington, R.; Karnosky, D. F. (2002) Altered performance of forest pests under atmospheres enriched with CO_2 and O_3. Nature (London) 420: 403-407.
Persson, K.; Danielsson, H.; Seldén, G.; Pleijel, H. (2003) The effects of tropospheric ozone and elevated carbon dioxide on potato (*Solanum tuberosum* L cv Bintje) growth and yield. Sci. Total Environ. 310: 191-201.
Peters, J. L.; Castillo, F. J.; Heath, R. L. (1988) Alteration of extracellular enzymes in pinto bean leaves upon exposure to air pollutants, ozone and sulfur dioxide. Plant Physiol. 89: 159-164.
Peterson, D. L.; Arbaugh, M. J.; Wakefield, V. A.; Miller, P. R. (1987) Evidence of growth reduction in ozone-injured Jeffrey pine (*Pinus jeffreyi* Grev and Balf) in Sequoia and Kings Canyon National Parks. JAPCA 37: 906-912.
Peterson, D. L.; Arbaugh, M. J.; Robinson, L. J. (1991) Regional growth changes in ozone-stressed ponderosa pine (*Pinus ponderosa*) in the Sierra Nevada, California, USA. Holocene 1: 50-61.
Peterson, D. L.; Arbaugh, M. J.; Robinson, L. J. (1993) Effects of ozone and climate on ponderosa pine (*Pinus ponderosa*) growth in the Colorado Rocky Mountains. Can. J. For. Res. 23: 1750-1759.
Peterson, D. L.; Silsbee, D. G.; Poth, M.; Arbaugh, M. J.; Biles, F. E. (1995) Growth response of bigcone Douglas fir (*Pseudotsuga macrocarpa*) to long-term ozone exposure in southern California. J. Air Waste Manage. Assoc. 45: 36-45.
Pfirrmann, T.; Barnes, J. D. (1993) Interactive effects of CO_2 and O_3 on growth of radish (*Raphanus sativus* L). Vegetatio 104-105: 464-466.
Phillips, R. L.; Zak, D. R.; Holmes, W. E.; White, D. C. (2002) Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131: 236-244.
Pimentel, D.; Wilson, C.; McCullum, C.; Huang, R.; Dwen, P.; Flack, J.; Tran, Q.; Saltman, T.; Cliff, B. (1997) Economic and environmental benefits of biodiversity. BioScience 47: 747-757.
Pimm, S. L. (1997) The value of everything. Nature (London) 387: 231-232.
Pino, M. E.; Mudd, J. B.; Bailey-Serres, J. (1995) Ozone-induced alterations in the accumulation of newly synthesized proteins in leaves of maize. Plant Physiol. 108: 777-785.
Pitcher, L. H.; Zilinskas, B. A. (1996) Overexpression of copper/zinc superoxide dismutase in the cytosol of transgenic tobacco confers partial resistance to ozone-induced foliar necrosis. Plant Physiol. 110: 583-588.
Pitelka, L. F. (1988) Evolutionary responses of plants to anthropogenic pollutants. Trends in Ecology and Evolution 3: 233-236.
Pleijel, H. (1998) A suggestion of a simple transfer function for the use of ozone monitoring data in dose-response relationships obtained using open-top chambers. Water Air Soil Pollut. 102: 61-74.
Pleijel, H.; Danielsson, H. (1997) Growth of 27 herbs and grasses in relation to ozone exposure and plant strategy. New Phytol. 135: 361-367.
Pleijel, H.; Skärby, L.; Ojanpera, K.; Selldén, G. (1994a) Exposure of oats, *Avena sativa* L, to filtered and unfiltered air in open-top chambers: Effects on grain yield and quality. Environ. Pollut. 86: 129-134.
Pleijel, H.; Wallin, G.; Karlsson, P. E.; Skärby, L.; Selldén, G. (1994b) Ozone deposition to an oat crop (*Avena sativa* L) grown in open-top chambers and in the ambient air. Atmos. Environ. 28: 1971-1979.

Pleijel, H.; Karlsson, G. P.; Danielsson, H.; Selldén, G. (1995a) Surface wetness enhances ozone deposition to a pasture canopy. Atmos. Environ. 29: 3391-3393.
Pleijel, H.; Wallin, G.; Karlsson, P.; Skärby, L.; Selldén, G. (1995b) Gradients of ozone at a forest site and over a field crop - consequences for the AOT4O concept of critical level. Water Air Soil Pollut. 85: 2033-2038.
Pleijel, H.; Karlsson, G. P.; Sild, E.; Danielsson, H.; Skärby, L.; Selldén, G. (1996) Exposure of a grass-clover mixture to ozone in open-top chambers–effects on yield, quality and botanical composition. Agric. Ecosyst. Environ. 59: 55-62.
Pleijel, H.; Ojanperä, K.; Mortensen, L. (1997) Effects of tropospheric ozone on the yield and grain protein content of spring wheat (*Triticum aestivum* L) in the nordic countries. Acta Agric. Scand. Sect. B 47: 20-25.
Pleijel, H.; Danielsson, H.; Gelang, J.; Sild, E.; Selldén, G. (1998) Growth stage dependence of the grain yield response to ozone in spring wheat (*Triticum aestivum* L). Agric. Ecosyst. Environ. 70: 61-68.
Pleijel, H.; Mortensen, L.; Fuhrer, J.; Ojanperä, K.; Danielsson, H. (1999a) Grain protein accumulation in relation to grain yield of spring wheat (*Triticum aestivum* L) grown in open-top chambers with different concentrations of ozone, carbon dioxide and water availability. Agric. Ecosyst. Environ. 72: 265-270.
Pleijel, H.; Norberg, P. A.; Selldén, G.; Skärby, L. (1999b) Tropospheric ozone decreases biomass production in radish plants (*Raphanus sativus*) grown in rural south-west Sweden. Environ. Pollut. 106: 143-147.
Pleijel, H.; Danielsson, H.; Karlsson, G. P.; Gelang, J.; Karlsson, P. E.; Selldén, G. (2000a) An ozone flux-response relationship for wheat. Environ. Pollut. 109: 453-462.
Pleijel, H.; Gelang, J.; Sild, E.; Danielsson, H.; Younis, S.; Karlsson, P. E.; Wallin, G.; Skärby, L.; Selldén, G. (2000b) Effects of elevated carbon dioxide, ozone and water availability on spring wheat growth and yield. Physiol. Plant. 108: 61-70.
Pleijel, H.; Danielsson, h.; Ojanperä, K.; De Temmerman, L.; Högy, P. (2002a) Relationships between ozone exposure and yield loss in wheat and potato - suggestions of critical levels for ozone effects on crops. In: Background Paper: Crops, workshop establishing ozone critical levels II; November; Göteborg, Sweden. Göteborg, Sweden: Göteborg University, IVL Swedish Environmental Research Institute; pp. 18-30.
Pleijel, H.; Danielsson, H.; Vandermeiren, K.; Blum, C.; Colls, J.; Ojanperä, K. (2002b) Stomatal conductance and ozone exposure in relation to potato tuber yield–results from the European CHIP programme. Eur. J. Agron. 17: 303-317.
Pleijel, H.; Danielsson, H.; Ojanpera, K.; De Temmerman, L.; Hogy, P.; Badiani, M.; Karlsson, P. E. (2004) Relationship between ozone exposure and yield loss in European wheat and potato–a comparison of concentration- and flux-based exposure indices. Atmos. Environ. 38: 2259-2269.
Plöchl, M.; Lyons, T.; Ollerenshaw, J.; Barnes, J. (2000) Simulating ozone detoxification in the leaf apoplast through the direct reaction with ascorbate. Planta 210: 454-467.
Polasky, S. ed. (2001) The economics of biodiversity conservation. Burlington, VT: Ashgate Publishers.
Polle, A. (1998) Photochemical oxidants: uptake and detoxification mechanisms. In: De Kok, L. J.; Stulen, I., eds. Responses of plant metabolism to air pollution and global change. Leiden, The Netherlands: Backhuys Publishers; pp. 95-116.
Polle, A.; Junkermann, W. (1994) Inhibition of apoplastic and symplastic peroxidase activity from Norway spruce by the photooxidant hydroxymethyl hydroperoxide. Plant Physiol. 104: 617-621.
Polle, A.; Pell, E. J. (1999) Role of carbon dioxide in modifying the plant response to ozone. In: Luo, Y.; Mooney, H. A., eds. Carbon dioxide and environmental stress. Academic Press; pp. 193-213.
Polle, A.; Rennenberg, H. (1991) Superoxide dismutase activity in needles of Scots pine and Norway spruce under field and changer conditions: lack of ozone effects. New Phytol. 117: 335-343.
Polle, A.; Krings, B.; Rennenberg, H. (1989) Superoxide dismutase activity in needles of Norwegian spruce trees (*Picea abies* l). Plant Physiol. 90: 1310-1315.
Poorter, H.; Pérez-Soba, M. (2001) The growth response of plants to elevated CO_2 under non-optimal environmental conditions. Oecologia 129: 1-20.
Posthumus, A. C. (1998) Air pollution and global change: significance and prospectives. In: De Kok, L. J.; Stulen, I., eds. Responses of plant metabolism to air pollution and global change. Leiden, The Netherlands: Backhuys Publishers; pp. 3-14.
Postiglione, L.; Fagnano, M.; Merola, G. (2000) Response to ambient ozone of two white clover (*Trifolium repens* Lcv"Regal") biotypes, one resistant and one sensitive, grown in a Mediterranean environment. Environ. Pollut. 109: 525-531.

Poth, M. A.; Fenn, M. E. (1998) Mature ponderosa pine nutrient use and allocation responses to air pollution. In: Bytnerowicz, A.; Arbaugh, M. J.; Schilling, S. L. Proceedings of the International Symposium on Air Pollution and Climate Change Effects on Forest Ecosystems; February, 1996; Riverside, CA. Albany, CA: USDA Forest Service, Pacific Southwest General Technical Report no. PSW-GTR 166; pp. 239-247.

Potter, L.; Foot, J. P.; Caporn, S. J. M.; Lee, J. A. (1996) The effects of long-term elevated ozone concentrations on the growth and photosynthesis of *Sphagnum recurvum* and *Polytrichum commune*. New Phytol. 134: 649-656.

Powell, M. C.; Muntifering, R. B.; Lin, J. C.; Chappelka, A. H. (2003) Yield and nutritive quality of sericea lespedeza (*Lespedeza cuneata*) and little bluestem (*Schizachyrium scoparium*) exposured to ground-level ozone. Environ. Pollut. 122: 313-322.

Powers, R. F. (1981) Nutritional ecology of Ponderosa pine (*Pinus ponderosa* L.) and associated species [dissertation]. Berkeley, CA: University of California.

Pronos, J.; Merrill, L.; Dahlsten, D. (1999) Insects and pathogens in a pollution-stressed forest. In: Miller, P. R.; McBride, J. R., eds. Oxidant air pollution impacts in the montane forests of southern California. Springer, pp. 317-337.

Prozherina, N.; Freiwald, V.; Rousi, M.; Oksanen, E. (2003) Interactive effect of springtime frost and elevated ozone on early growth, foliar injuries and leaf structure of birch (*Betula pendula*). New Phytol. 159: 623-636.

Pryor, W. A. (1992) How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? Free Radic. Biol. Med. 12: 83-88.

Qui, Z.; Chappelka, A. H.; Somers, G. L.; Lockaby, B. G.; Meldahl, R. S. (1993) Effects of ozone and simulated acidic precipitation on ectomycorrhizal formation on loblolly pine seedlings. Environ. Exp. Bot. 33: 423-431.

Rabinowitch, E. I. (1945) Photosynthesis and related processes Vol 1: chemistry of photosynthesis, chemosynthesis and related processes in vitro and in vivo. New York, NY: Interscience Publishing, Inc..

Rafarel, C. R.; Ashenden, T. W. (1991) A facility for the large-scale exposure of plants to gaseous atmospheric pollutants. New Phytol. 117: 345-349.

Randall, A. (1991) The value of biodiversity. Ambio 20: 64-68.

Rangnekar, P. V.; Forward, D. F.; Nolan, N. J. (1969) Foliar nutrition and wood growth in red pine: the distribution of radiocarbon photoassimilated by individual branches of young trees. Can. J. Bot. 47: 1701-1711.

Rantanen, L.; Palomäki, V.; Holopainen, T. (1994) Interactions between exposure to O_3 and nutrient status of trees: effects on nutrient content and uptake, growth, mycorrhiza and needle ultrastructure. New Phytol. 128: 679-687.

Rao, M.; Davis, K. R. (1999) Ozone-induced cell death occurs via two distinct mechanisms in *Arabidopsis*: the role of salicylic acid. Plant J. 17: 603-614.

Rao, M. V.; Davis, K. R. (2001) The physiology of ozone induced cell death. Planta 213: 682-690.

Rao, M. V.; Hale, B. A.; Ormrod, D. P. (1995) Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide Role of antioxidant enzymes. Plant Physiol. 109: 421-432.

Rao, M. V.; Lee, H. I.; Davis, K. R. (2002) Ozone-induced ethylene production is dependent on salicylic acid, and both salicylic acid and ethylene act in concert to regulate ozone-induced cell death. Plant J. 32: 447-456.

Read, J. J.; Morgan, J. A. (1996) Growth and partitioning in *Pascopyrum smithii* (C3) and *Bouteloua gracilis* (C4) as influenced by carbon dioxide and temperature. Ann. Bot. (London, UK) 77: 487-496.

Reams, G. A.; Van Deusen, P. C.; Lucier, A. A. (1995) Ambient ozone and loblolly pines. Nature (London) 378: 449-450.

Rebbeck, J. (1996) Chronic ozone effects on three northeastern hardwood species: growth and biomass. Can. J. For. Res. 26: 1788-1798.

Rebbeck, J.; Scherzer, A. J. (2002) Growth responses of yellow-poplar (*Liriodendron tulipifera* L.) exposed to 5 years of O_3 alone or combined with elevated CO_2. Plant Cell Environ. 25: 1527-1537.

Rebbeck, J.; Blum, U.; Heagle, A. S. (1988) Effects of ozone on the regrowth and energy reserves of a ladino clover-tall fescue pasture. J. Appl. Ecol. 25: 659-681.

Rebbeck, J.; Jensen, K. F.; Greenwood, M. S. (1993) Ozone effects on grafted mature and juvenile red spruce: photosynthesis, stomatal conductance, and chlorophyll concentration. Can. J. For. Res. 23: 450-456.

Reddy, G. B.; Reinert, R. A.; Eason, G. (1991) Effect of acid rain and ozone on soil and secondary needle nutrients of loblolly pine. In: Wright, R. J.; Baligar, V. C.; Murrmann, R. P., eds. Plant-soil interactions at low pH: proceedings of the second international symposium; June 1990; Beckley, WV. Dordrecht, The Netherlands: Kluwer Academic Publishers; pp. 139-145.

Reddy, G. N.; Arteca, R. N.; Dai, Y. R.; Flores, H. E.; Negm, F. B.; Pell, E. J. (1993) Changes in ethylene and polyamines in relation to mRNA levels of the large and small subunits of ribulose bisphosphate carboxylase/oxygenase in ozone-stressed potato foliage. Plant Cell Environ. 16: 819-826.

Reich, P. B. (1983) Effects of low concentrations of O_3 on net photosynthesis, dark respiration, and chlorophyll contents in aging hybrid poplar leaves. Plant Physiol. 73: 291-296.
Reich, P. B. (1987) Quantifying plant response to ozone: a unifying theory. Tree Physiol. 3: 63-91.
Reich, P. B. (2002) Root-shoot relations: optimality in acclimation and adaptation or the "Emperor's New Clothes"? In: Waisel, Y.; Eshel, A.; Kafkafi, U., eds. Plant roots - the hidden half. Marcel Dekker; pp. 205-220.
Reich, P. B.; Lassoie, J. P. (1984) Effects of low level O_3 exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. Plant Cell Environ. 7: 661-668.
Reich, P. B.; Schoettle, A. W.; Stroo, H. F.; Troiano, J.; Amundson, R. G. (1985) Effects of O_3, SO_2, and acidic rain on mycorrhizal infection in northern red oak seedlings. Can. J. Bot. 63: 2049-2055.
Reichenauer, T. G.; Goodman, B. A.; Kostecki, P.; Soja, G. (1998) Ozone sensitivity in *Triticum durum* and *T. aestivum* with respect to leaf injury, photosynthetic activity and free radical content. Physiol. Plant. 104: 681-686.
Reid, C. D.; Fiscus, E. L. (1998) Effects of elevated [CO_2] and/or ozone on limitations to CO_2 assimilation in soybean (*Glycine max*). J. Exp. Bot. 49: 885-895.
Reid, C. D.; Fiscus, E. L.; Burkey, K. O. (1998) Combined effects of chronic ozone and elevated CO_2 on rubisco activity and leaf components in soybean (*Glycine max*). J. Exp. Bot. 49: 1999-2011.
Reiling, K.; Davison, A. W. (1992a) Effects of a short ozone exposure given at different stages in the development of *Plantago major* L. New Phytol. 121: 643-647.
Reiling, K.; Davison, A. W. (1992b) The response of native, herbaceous species to ozone: growth and fluorescence screening. New Phytol. 120: 29-37.
Reiling, K.; Davison, A. W. (1992c) Spatial variation in ozone resistance of British populations of *Plantago major* L. New Phytol. 122: 699-708.
Reiling, K.; Davison, A. W. (1994) Effects of exposure to ozone at different stages in the development of *Plantago major* L on chlorophyll fluorescence and gas exchange. New Phytol. 128: 509-514.
Reiner, S.; Wiltshire, J. J. J.; Wright, C. J.; Colls, J. J. (1996) The impact of ozone and drought on the water relations of ash trees (*Fraxinus excelsior* L). J. Plant Physiol. 148: 166-171.
Reinert, R. A.; Ho, M. C. (1995) Vegetative growth of soybean as affected by elevated carbon dioxide and ozone. Environ. Pollut. 89: 89-96.
Reinert, R. A.; Eason, G.; Barton, J. (1997) Growth and fruiting of tomato as influenced by elevated carbon dioxide and ozone. New Phytol. 137: 411-420.
Renaud, J. P.; Allard, G.; Mauffette, Y. (1997) Effects of ozone on yield, growth, and root starch concentrations of two alfalfa (*Medicago sativa* L) cultivars. Environ. Pollut. 95: 273-281.
Rennenberg, H.; Herschbach, C.; Polle, A. (1996) Consequences of air pollution on shoot-root interactions. J. Plant Physiol. 148: 296-301.
Retzlaff, W. A.; Weinstein, D. A.; Laurence, J. A.; Gollands, B. (1996) Simulated root dynamics of a 160-year-old sugar maple (*Acer saccharum* Marsh) tree with and without ozone exposure using the TREGRO model. Tree Physiol. 16: 915-921.
Retzlaff, W. A.; Williams, L. E.; DeJong, T. M. (1997) Growth and yield response of commercial bearing-age 'Casselman' plum trees to various ozone partial pressures. J. Environ. Qual. 26: 858-865.
Retzlaff, W. A.; Arthur, M. A.; Grulke, N. E.; Weinstein, D. A.; Gollands, B. (2000) Use of a single-tree simulation model to predict effects of ozone and drought on growth of a white fir tree. Tree Physiol. 20: 195-202.
Ribas, A.; Peñuelas, J. (2000) Effects of ethylene diurea as a protective antiozonant on beans (*Phaseolus vulgaris* cv lit) exposed to different tropospheric ozone doses in Catalonia (NE Spain). Water Air Soil Pollut. 117: 263-271.
Rich, S.; Ames, R.; Zuckel, J. W. (1974) 1,4-oxanthin derivatives protect plants against ozone. Plant Dis. Rep. 58: 162-164.
Ritchie, G. A.; Dunlap, J. R. (1980) Root growth potential: its development and expression in forest tree seedlings. N. Z. J. For. Sci. 10: 218-248.
Robinson, J. M.; Britz, S. J. (2000) Tolerance of a field grown soybean cultivar to elevated ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydroascorbate redox status, and long term photosynthetic productivity. Photosynth. Res. 64: 77-87.
Robinson, M. F.; Heath, J.; Mansfield, T. A. (1998) Disturbance in stomatal behaviour caused by air pollutants. J. Exp. Bot. 49: 461-469.

Rogers, A.; Allen, D. J.; Davey, P. A.; Morgan, P. B.; Ainsworth, E. A.; Bernacchi, C. J.; Cornic, G.; Dermody, O. C.; Heaton, E. A.; Mahoney, J.; Zhu, X. G.; DeLucia, E. H.; Ort, D. R.; Long, S. P. (2004) Leaf photosynthesis and carbohydrate dynamics of soybean grown throughout their life-cycle under free-air carbon dioxide enrichment. Plant Cell Environ. 28: 449-458.

Roitsch, T. (1999) Source-sink regulation by sugar and stress. Curr. Opin. Plant Biol. 2: 198-206.

Rondön, A.; Johansson, C.; Granat, L. (1993) Dry deposition of nitrogen-dioxide and ozone to coniferous forests. J. Geophys. Res. [Atmos.] 98(D3): 5159-5172.

Rosen, P. M.; Runeckles, V. C. (1976) Interaction of ozone and greenhouse whitefly in plant injury. Environ. Conserv. 3: 70-71.

Roth, D. R.; Fahey, T. J. (1998) The effects of acid precipitation and ozone on the ectomycorrhizae of red spruce saplings. Water Air Soil Pollut. 103: 263-276.

Rötter, R.; Van De Geijn, S. C. (1999) Climate change effects on plant growth, crop yield and livestock. Clim. Change 43: 651-681.

Rowland-Bamford, A. J. (2000) Plant responses to changing carbon dioxide and temperature. In: Singh, S. N., ed. Trace gas emissions and plants. Dordrecht, The Netherlands: Kluwer Academic Publishers; pp. 63-74.

Rudorff, B. F. T.; Mulchi, C. L.; Daughtry, C. S. T.; Lee, E. H. (1996a) Growth, radiation use efficiency, and canopy reflectance of wheat and corn grown under elevated ozone and carbon dioxide atmospheres. Remote Sens. Environ. 55: 163-173.

Rudorff, B. F. T.; Mulchi, C. L.; Fenny, P.; Lee, E. H.; Rowland, R. (1996b) Wheat grain quality under enhanced tropospheric CO_2 and O_3 concentrations. J. Environ. Qual. 25: 1384-1388.

Rudorff, B. F. T.; Mulchi, C. L.; Lee, E. H.; Rowland, R.; Pausch, R. (1996c) Effects of enhanced O_3 and CO_2 enrichment on plant characteristics in wheat and corn. Environ. Pollut. 94: 53-60.

Rudorff, B. F. T.; Mulchi, C. L.; Lee, E. H. (2000) Plant responses to elevated CO_2 and interactions with O_3. In: Singh, S. N., ed. Trace gas emissions and plants. Dordrecht, The Netherlands: Kluwer Academic Publishers; pp. 155-179.

Runeckles, V. C. (1974) Dosage of air pollutants and damage to vegetation. Environ. Conserv. 1: 305-308.

Runeckles, V. C. (2002) Air pollution and climate change. In: Bell, J. N. B.; Treshow, M., eds. Air pollution and plant life. Chichester, United Kingdom: John Wiley & Sons Ltd.; pp. 431-454.

Runeckles, V. C.; Bowen, P. A. (2000) The use of calibrated passive monitors to assess crop loss due to ozone in rural locations. In: Agrawal, S. B.; Agrawal, M., eds. Environmental pollution and plant response. Boca Raton, Florida: Lewis Publishers; pp. 307-316.

Runeckles, V. C.; Krupa, S. V. (1994) The impact of UV-B radiation and ozone on terrestrial vegetation. Environ. Pollut. 83: 191-213.

Runeckles, V. C.; Palmer, K. (1987) Pretreatment with nitrogen dioxide modifies plant response to ozone. Atmos. Environ. 21: 717-719.

Runeckles, V. C.; Vaartnou, M. (1997) EPR evidence for superoxide anion formation in leaves during exposure to low levels of ozone. Plant Cell Environ. 20: 306-314.

Runeckles, V. C.; Wright, E. F. (1996) Delayed impact of chronic ozone stress on young Douglas-fir grown under field conditions. Can. J. For. Res. 26: 629-638.

Runeckles, V. C.; Wright, E. F.; White, D. (1990) A chamberless field exposure system for determining the effects of gaseous air pollutants on crop growth and yield. Environ. Pollut. 63: 61-77.

Rusch, H.; Laurence, J. A. (1993) Interactive effects of ozone and powdery mildew on pea seedlings. Phytopathology 83: 1258-1263.

Sah, D. N.; Von Tiedemann, A.; Fehrmann, H. (1993) Effects of ozone exposure on resistance of wheat genotypes to *Phrenophora tritici-repentis*. J. Phytopathol. 138: 249-256.

Saitanis, C. J.; Karandinos, M. G. (2001) Instrumental recording and biomonitoring of ambient ozone in the Greek countryside. Chemosphere 44: 813-821.

Sakaki, T.; Kondo, N.; Sugahara, K. (1983) Breakdown of photosynthetic pigments and lipids in spinach leaves with ozone fumigation: role of active oxygens. Physiol. Plant. 59: 28-34.

Sakaki, T.; Ohnishi, J.-I.; Kondo, N.; Yamada, M. (1985) Polar and neutral lipid changes in spinach leaves with ozone fumigation: triacylglyerol synthesis from polar lipids. Plant Cell Physiol. 26: 253-262.

Sakaki, T.; Saito, K.; Kawaguchi, A.; Kondo, N.; Yamada, M. (1990) Conversion of monogalactosylidacyglyerol to triacylglycerols in ozone-fumigated spinach leaves. Plant Physiol. 94: 766-772.

Salardino, D. H.; Carroll, J. J. (1998) Correlation between ozone exposure and visible foliar injury in ponderosa and Jeffrey pines. Atmos. Environ. 32: 3001-3010.

Saleem, A.; Loponen, J.; Pihlaja, K.; Oksanen, E. (2001) Effects of long-term open-field ozone exposure on leaf phenolics of European silver birch (Betula pendula Roth). J. Chem. Ecol. 27: 1049-1062.
Samuelson, P. A. (1948) Economics: an introductory analysis. New York, NY: McGraw-Hill.
Samuelson, L. J. (1994) Ozone-exposure responses of black cherry and red maple seedlings. Environ. Exp. Bot. 34: 355-362.
Samuelson, L. J.; Edwards, G. S. (1993) A comparison of sensitivity to ozone in seedlings and trees of Quercus rubra L. New Phytol. 125: 373-379.
Samuelson, L. J.; Kelly, J. M. (1996) Carbon partitioning and allocation in northern red oak seedlings and mature trees in response to ozone. Tree Physiol. 16: 853-858.
Samuelson, L. J.; Kelly, J. M. (1997) Ozone uptake in *Prunus serotina*, *Acer rubrum* and *Quercus rubra* forest trees of different sizes. New Phytol. 136: 255-264.
Samuelson, L.; Kelly, J. M. (2001) Scaling ozone effects from seedlings to forest trees Tansley review no 21. New Phytol. 149: 21-41.
Samuelson, L. J.; Kelly, J. M.; Mays, P. A.; Edwards, G. S. (1996) Growth and nutrition of *Quercus rubra* L seedlings and mature trees after three seasons of ozone exposure. Environ. Pollut. 91: 317-323.
Sandermann, H., Jr. (1996) Ozone and plant health. Annu. Rev. Phytopathol. 34: 347-366.
Sandermann, H., Jr. (1998) Ozone: an air pollutant acting as a plant-signaling molecule. Naturwissenschaften 85: 369-375.
Sandermann, H., Jr. (2000) Ozone/biotic disease interactions: molecular biomarkers as a new experimental tool. Environ. Pollut. 108: 327-332.
Sasek, T. W.; Richardson, C. J.; Fendick, E. A.; Bevington, S. R.; Kress, L. W. (1991) Carryover effects of acid rain and ozone on the physiology of multiple flushes of loblolly pine seedlings. For. Sci. 37: 1078-1098.
Saxe, H.; Ellsworth, D. S.; Heath, J. (1998) Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol. 139: 395-436.
Sayre, R. G.; Fahey, T. J. (1999) Effects of rainfall acidity and ozone on foliar leaching in red spruce Picea rubens. Can. J. For. Res. 29: 486-496.
Scagel, C. F.; Andersen, C. P. (1997) Seasonal changes in root and soil respiration of ozone-exposed ponderosa pine (*Pinus ponderosa*) grown in different substrates. New Phytol. 136: 627-643.
Scandalios, J. G. (1993) Oxygen stress and superoxide dismutases. Plant Physiol. 101: 7-12.
Schaub, M.; Skelly, J. M.; Steiner, K. C.; Davis, D. D.; Pennypacker, S. P.; Zhang, J.; Ferdinand, J. A.; Savage, J. E.; Stevenson, R. E. (2003) Physiological and foliar injury responses of *Prunus serotina*, *Fraxinus americana*, and *Acer rubrum* seedlings to varying soil moisture and ozone. Environ. Pollut. 124: 307-320.
Scherzer, A. J.; Rebbeck, J.; Boerner, R. E. J. (1998) Folia nitrogen dynamics and decomposition of yellow-poplar and eastern white pine during four seasons of exposure to elevated ozone and carbon dioxide. For. Ecol. Manage. 109: 355-366.
Schmieden, U.; Wild, A. (1995) The contribution of ozone to forest decline. Physiol. Plant. 94: 371-378.
Schnitzler, J. P.; Langebartels, C.; Heller, W.; Liu, J.; Lippert, M.; Dohring, T.; Bahnweg, G.; Sandermann, H. (1999) Ameliorating effect of UV-B radiation on the response of Norway spruce and Scots pine to ambient ozone concentrations. Global Change Biol. 5: 83-94.
Schnug, E. (1998) Response of plant metabolism to air pollution and global change - impact on agriculture. In: De Kok, L. J. ; Stulen, I. , eds. Responses of plant metabolism to air pollution and global change. Leiden, The Netherlands: Backhuys Publishers; pp. 15-22.: De-22.
Schopfer, P. (1994) Histochemical demonstration and localization of H2O2 in organs of higher plants by tissue printing on nitrocellulose paper. Plant Physiol. 104: 1269-1275.
Schraudner, M.; Ernst, D.; Langebartels, C.; Sandermann, H., Jr. (1992) Biochemical plant responses to ozone III Activation of the defense-related proteins β-1,3-glucanase and chitinase in tobacco leaves. Plant Physiol. 99: 1321-1328.
Schraudner, M.; Langebartels, C.; Sandermann, H., Jr. (1996) Plant defence systems and ozone. Biochem. Soc. Trans. 24: 456-461.
Schraudner, M.; Langebartels, C.; Sandermann, H. (1997) Changes in the biochemical status of plant cells linduced by the environmental pollutant ozone. Physiol. Plant. 100: 274-280.
Schraudner, M.; Moeder, W.; Wiese, C.; Van Camp, W.; Inzé, D.; Langebartels, C.; Sandermann, H., Jr. (1998) Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco Bel W3. Plant J. 16: 235-245.
Schulte-Hosted, S.; Darrall, N. M.; Blank, L. W.; Wellburn, A. R. (1988) Air pollution and plant metabolism. New York, NY: Elsivier Applied Science.

Schweizer, B.; Arndt, U. (1990) CO_2/H2O gas exchange parameters of one- and two-year old needles of spruce and fir. Environ. Pollut. 68: 275-292.
Segerson, K. (1987) Impacts of ozone and acid rain: discussion. Am. J. Agric. Econ. 69: 970-971.
Segerson, K. (1991) Air pollution and agriculture: a review and evaluation of policy interactions. In: Just, R. E.; Bockstael, N., eds. Commodity and resource policies in agricultural systems. Springer-Verlag; pp. 349-367.
Seufert, G.; Arndt, U. (1985) Open-top-Kammern als Teil eines Konzepts zur ökosystemaren Untersuchung der neuartigen Waldschaden. Alg. Forstz. 40: 13-18.
Shafer, S. R. (1988) Influence of ozone and simulated acidic rain on microorganisms in the rhizosphere of *Sorghum*. Environ. Pollut. 51: 131-152.
Shan, Y.; Feng, Z.; Izuta, T.; Aoki, M.; Totsuka, T. (1996) The individual and combined effects of ozone and simulated acid rain on growth, gas exchange and water-use efficiency of *Pinus armandi* Franch. Environ. Pollut. 91: 355-361.
Sharma, Y. K.; Davis, K. R. (1995) Isolation of a novel *Arabidopsis* ozone-induced cDNA by differential display. Plant Mol. Biol. 29: 91-98.
Sharma, P.; Sôber, A.; Sôber, J.; Podila, G. K.; Kubiske, M. E.; Mattson, W. J.; Isebrands, J. G.; Karnosky, D. F. (2003) Moderation of [CO_2]-induced gas exchange responses by elevated tropospheric O_3 in trembling aspen and sugar maple. Ekologia (Bratislava): in press.
Shaw, M. R.; Zavaleta, E. S.; Chiariello, N. R.; Cleland, E. E.; Mooney, H. A.; Field, C. B. (2002) Grassland responses to global environmental changes suppressed by elevated CO_2. Science (Washington, DC, U. S.) 298: 1987-1990.
Sheng, Y.; Podila, G. K.; Karnosky, D. F. (1997) Differences in O_3-induced superoxide dismutase and glutathione antioxidant expression in O_3 tolerant and sensitive trembling aspen (*Populus tremuloides* Michx) clones. For. Gen. 4: 25-33.
Sheppard, L. J.; Cape, J. N. (1999) Forest growth responses to the pollution climate of the 21st century: proceedings of the 18th IUFRO workshop on air pollution stress; 1998; Edinburgh, Scotland. Water Air Soil Pollut. 116(1-2 special issue).
Sheppard, L. J.; Smith, R. I.; Cannell, M. G. R. (1989) Frost hardiness of Picea rubens growing in spruce decline regions of the Appalachians. Tree Physiol. 5: 25-37.
Shinshi, H.; Mohnen, D.; Meins, F., Jr. (1987) Regulation of a plant pathogenesis-related enzyme: inhibition of chitinase and chitinase mRNA accumulation in cultured tobacco tissues by auxin and cytokinin. Proc. Natl. Acad. Sci. U. S. A. 84: 89-93.
Shinshi, H.; Wenzler, H.; Neuhaus, J. M.; Felix, G.; Hofsteenge, J.; Meins, F., Jr. (1988) Evidence for N- and C-terminal processing of a plant defense-related enzyme: primary structure of tobacco prepro-β-1,3-glucanase. Proc. Natl. Acad. Sci. U. S. A. 85: 5541-5545.
Sild, E.; Pleijel, H.; Selldén, G. (1999) Response of subterranean clover (*Trifolium subterraneum*) to ozone in relation to plant age and light conditions during exposure. New Phytol. 143: 315-321.
Simini, M.; Skelly, J. M.; Davis, D. D.; Savage, J. E.; Comrie, A. C. (1992) Sensitivity of four hardwood species to ambient ozone in north central Pennsylvania. Can. J. For. Res. 22: 1789-1799.
Simmons, G. L.; Kelly, J. M. (1989) Influence of O_3, rainfall acidity, and soil Mg status on growth and ectomycorrhizal colonization of loblolly pine roots. Water Air Soil Pollut. 44: 159-171.
Simon-Plas, F.; Rustérucci, C.; Milat, M. L.; Humbert, C.; Montillet, J. L.; Blein, J. P. (1997) Active oxygen species production in tobacco cells elicited by cryptogein. Plant Cell Environ. 20: 1573-1579.
Simpson, D.; Tuovinen, J.-P.; Emberson, L.; Ashmore, M. R. (2001) Characteristics of an ozone deposition module. Water Air Soil Pollut. Focus 1: 253-262.
Simpson, D.; Tuovinen, J.-P.; Emberson, L.; Ashmore, M. R. (2003) Characteristics of an ozone deposition module II: Sensitivity analysis. Water Air Soil Pollut. 143: 123-137.
Singh, S. N. ed. (2000) Trace gas emissions and plants. Dordrecht, The Netherlands: Kluwer Academic Publishers, 368 pp.
Sivasankar, S.; Sheldrick, B.; Rothstein, S. J. (2000) Expression of allene oxide synthase determines defense gene activation in tomato. Plant Physiol. 122: 1335-1342.
Skärby, L.; Ro-Poulsen, H.; Wellburn, F. A. M.; Sheppard, L. J. (1998) Impacts of ozone on forests: a European perspective. New Phytol. 139: 109-122.
Skärby, L.; Ottosson, S.; Karlsson, P. E.; Wallina, G.; Selldén, G.; Medina, E. L.; Pleijel, H. (2004) Growth of Norway spruce (*Picea abies*) in relation to different ozone exposure indices: a synthesis. Atmos. Environ. 38: 2225-2236.

Skeffington, R. A.; Sutherland, P. M. (1995) The effects of SO_2 and O_3 fumigation on acid deposition and foliar leaching in the Liphook forest fumigation experiment. Plant Cell Environ. 18: 247-261.
Skelly, J. M. (1988) Forest decline versus tree decline the pathological considerations. Environ. Monit. Assess. 12: 23-27.
Skelly, J. M. (2003) Native plants as bioindicators of air pollutants: contributed papers to a symposium held in conjunction with the 34th air pollution workshop. Environ. Pollut. 125: 1-2.
Smeulders, S. M.; Gorissen, A.; Joosten, N. N.; Van Veen, J. A. (1995) Effects of short-term ozone exposure on the carbon economy of mature and juvenile Douglas firs [*Pseudotsuga menziesii* (Mirb.) Franco]. New Phytol. 129: 45-53.
Smirnoff, N.; Conklin, P. L.; Loewus, F. A. (2001) Biosynthesis of ascorbate acid in plants: a renaissance. Annu. Rev. Plant Physiol. Mol. Biol. 52: 437-467.
Smith, W. H. (1992) Air pollution effects on ecosystem processes. In: Barker, J. R.; Tingey, D. T., eds. Air pollution effects on biodiversity. New York, NY: Van Nostrand Reinhold; pp. 234-260.
Smith, W. B. (2002) Forest inventory and analysis: a national inventory and monitoring program. Environ. Pollut. 116: S233-S242.
Smith, S. E.; Read, D. J. (1997) Mycorrhizal symbiosis. San Diego, CA: Academic Press.
Smith, G.; Coulston, J.; Jepsen, E.; Prichard, T. (2003) A national ozone biomonitoring program - results from field surveys of ozone sensitive plants in northeastern forests (1994-2000). Environ. Monit. Assess. 87: 271-291.
Soja, G.; Eid, M.; Gangl, H.; RedI, H. (1997) Ozone sensitivity of grapevine (*Vitis vinifera* L): evidence for a memory effect in a perennial crop plant? Phyton (Austria) 37: 265-270.
Soja, G.; Barnes, J. D.; Posch, M.; Vandermeiren, K.; Pleijel, H.; Mills, G. (2000) Phenological weighting of ozone exposures in the calculation of critical levels for wheat, bean and plantain. Environ. Pollut. 109: 517-524.
Soja, G.; Reichenauer, T. G.; Eid, M.; Soja, A. M.; Schaber, R.; Gangl, H. (2004) Long-term ozone exposure and ozone uptake of grapevines in open-top chambers. Atmos. Environ. 38: 2313-2321.
Somers, G. L.; Chappelka, A. H.; Rosseau, P.; Renfro, J. R. (1998) Empirical evidence of growth decline related to visible ozone injury. For. Ecol. Manage. 104: 129-137.
Spash, C. L. (1997) Assessing the economic benefits to agriculture from air pollution control. J. Econ. Surv. 11: 47-70.
Spence, R. D.; Rykiel, E. J., Jr.; Sharpe, P. J. H. (1990) Ozone alters carbon allocation in loblolly pine: assessment with carbon-11 labeling. Environ. Pollut. 64: 93-106.
Stan, H. J.; Schicker, S. (1982) Effect of repetitive ozone treatment on bean plants - stress ethylene production and leaf necrosis. Atmos. Environ. 16: 2267-2270.
Stan, H. J.; Schicker, S.; Kassner, H. (1981) Stress ethylene evolution of bean plants - a parameter indicating ozone pollution. Atmos. Environ. 15: 391-395.
Stark, R. W.; Cobb, F. W., Jr. (1969) Smog injury, root diseases and bark beetle damage in ponderosa pine. Calif. Agric. 23: 13-15.
Stark, R. W.; Miller, P. R.; Cobb, F. W., Jr.; Wood, D. L.; Parmeter, J. R., Jr. (1968) Photochemical oxidant injury and bark beetle (Coleoptera: Scolytidae) infestation of ponderosa pine I. Incidence of bark beetle infestation in injured trees. Hilgardia 39: 121-126.
Staszak, J.; Grulke, N. E.; Prus-Glowacki, W. (2004) Genetic differences of *Pinus ponderosa* (Dougl Ex Laws) trees tolerant and sensitive to ozone. Water Air Soil Pollut. 153: 3-14.
Stewart, C. A. (1998) Impact of ozone on the reproductive biology of *Brassica campestris* L. and *Plantago major* L [dissertation]. Leicestershire, United Kingdom: University of Loughborough, Department of Geology.
Stewart, C. A.; Black, V. J.; Black, C. R.; Roberts, J. A. (1996) Direct effects of ozone on the reproductive development of *Brassica* species. J. Plant Physiol. 148: 172-178.
Stitt, M. (1996) Plasmodesmata play an essential role in sucrose export from leaves: a step towards an integration of metabolic biochemistry and cell biology. Plant Cell 8: 565-571.
Stolzy, L. H.; Taylor, O. C.; Dugger, W. M., Jr.; Mersereau, J. D. (1964) Physiological changes in and ozone susceptibility of the tomato plant after short periods of inadequate oxygen diffusion to the roots. Soil Sci. Soc. Am. Proc. 28: 305-308.
Stow, T. K.; Allen, H. L.; Kress, L. W. (1992) Ozone impacts on seasonal foliage dynamics of young loblolly pine. For. Sci. 38: 102-119.
Stribley, G. H.; Ashmore, M. R. (2002) Quantitative changes in twig growth pattern of young woodland beech (*Fagus sylvatica* L.) in relation to climate and ozone pollution over 10 years. For. Ecol. Manage. 157: 191-204.

Strohm, M.; Eiblmeier, M.; Langebartels, C.; Jouanin, L.; Polle, A.; Sandermann, H.; Rennenberg, H. (1999) Responses of transgenic poplar (*Populus tremula* x *P. alba*) overexpressing glutathione synthetase of glutathione reductase to acute ozone stress: visible injury and leaf gas exchange. J. Exp. Bot. 50: 363-372.

Strohm, M.; Eiblmeier, M.; Langebartels, C.; Jouanin, L.; Polle, A.; Sandermann, H.; Rennenberg, H. (2002) Responses of antioxidative systems to acute ozone stress in transgenic poplar (*Populus tremula* x *P. alba*) over-expressing glutathione synthetase or glutathione reductase. Trees (Heidelberg, Ger.) 16: 262-273.

Stroo, H. F.; Reich, P. B.; Schoettle, A. W.; Amundson, R. G. (1988) Effects of ozone and acid rain on white pine (*Pinus strobus*) seedlings grown in five soils II Mycorrhizal infection. Can. J. Bot. 66: 1510-1516.

Suter, G. W., II (1990) Endpoints for regional ecological risk assessments. Environ. Manage. (N. Y.) 14: 9-23.

Swank, W. T.; Vose, J. M. (1990) Watershed-scale responses to ozone events in a *Pinus strobus* L plantation. Water Air Soil Pollut. 54: 119-133.

Szaro, R.; Bytnerowicz, A.; Oszlányi, J. (2002) Effects of air pollution on forest health and biodiversity in forests of the Carpathian Mountains. Amsterdam, The Netherlands: IOS Press.

Taiz, L.; Zeiger, E. (2002) Plant physiology, 3rd ed. Sunderland, MA: Sinauer Associates, Publishers.

Takemoto, B. K.; Olszyk, D. M.; Johnson, A. G.; Parada, C. R. (1988) Yield responses of field-gown crops to acidic fog and ambient ozone. J. Environ. Qual. 17: 192-197.

Takemoto, B. K.; Bytnerowicz, A.; Dawson, P. J.; Morrison, C. L.; Temple, P. J. (1997) Effects of ozone on *Pinus ponderosa* seedlings: comparison of responses in the first and second growing seasons of exposure. Can. J. For. Res. 27: 23-30.

Takemoto, B. K.; Bytnerowicz, A.; Fenn, M. E. (2001) Current and future effects of ozone and atmospheric nitrogen deposition on California's mixed conifer forests. For. Ecol. Manage. 144: 159-173.

Takeuchi, Y.; Kubiske, M. E.; Isebrands, J. G.; Pregitzer, K. S.; Hendrey, G.; Karnosky, D. F. (2001) Photosynthesis, light and nitrogen relationships in a young deciduous forest canopy under open-air CO_2 enrichment. Plant Cell Environ. 24: 1257-1268.

Tamaoki, M.; Nakajima, N.; Kubo, A.; Aono, M.; matsuyama, T.; Saji, H. (2003) Transcriptome analysis of O_3-exposed Arabidopsis reveals that multiple signal pathways act mutually antagonistically to induce gene expression. Plant Mol. Biol. 53: 443-456.

Tappel, A. L. (1972) Vitamin E and free radical peroxidation of lipids. In: Nair, P. P.; Kayden, H. J., eds. Vitamin E and its role in cellular metabolism. New York, NY: New York Academy of Sciences; pp. 12-28. (Annals of the New York Academy of Sciences: v. 203).

Tausz, M.; Bytnerowicz, A.; Arbaugh, M. J.; Weidner, W.; Grill, D. (1999a) Antioxidants and protective pigments of *Pinus ponderosa* needles at gradients of natural stresses and ozone in the San Bernardino Mountains in California. Free Rad. Res. 31(suppl.): S113-S120.

Tausz, M.; Bytnerowicz, A.; Weidner, W.; Arbaugh, M. J.; Padgett, P.; Grill, D. (1999b) Changes in free-radical scavengers describe the susceptibility of *Pinus ponderosa* to ozone in southern Californian forests. Water Air Soil Pollut. 116: 249-254.

Tausz, M.; Bytnerowicz, A.; Weidner, W.; Arbaugh, M. J.; Padgett, P.; Grill, D. (1999c) Pigments and phytoprotection in needles of *Pinus ponderosa* trees with and without symptoms of ozone injury. Phyton (Horn, Austria) 39: 219-224.

Tausz, M.; Bytnerowicz, A.; Arbaugh, M. J.; Wonisch, A.; Grill, D. (2001) Multivariate patterns of biochemical responses of *Pinus ponderosa* trees at field plots in the San Bernardino Mountains, southern California. Tree Physiol. 21: 329-336.

Tausz, M.; Herbinger, K.; Posch, S.; Grulke, N. E. (2002) Antioxidant status of *Pinus jeffreyi* needles from mesic and xeric microsites in early and late summer. Phyton (Horn, Austria) 42: 201-207.

Taylor, G. E., Jr.; Hanson, P. J. (1992) Forest trees and tropospheric ozone: role of canopy deposition and leaf uptake in developing exposure-response relationships. Agric. Ecosyst. Environ. 42: 255-273.

Taylor, G. E., Jr.; Pitelka, L. F. (1992) Genetic diversity of plant populations and the role of air pollution. In: Barker, J. R.; Tingey, D. T., eds. Air pollution effects on biodiversity. Van Nostrand Reinhold; pp. 111-130.

Taylor, G. E., Jr.; McLaughlin, S. B.; Shriner, D. S. (1982) Effective pollutant dose. In: Unsworth, M. H.; Ormrod, D. P., eds. Effects of gaseous air pollution in agriculture and horticulture. London, United Kingdom: Butterworth Scientific; pp. 458-460.

Taylor, G. E., Jr.; Tingey, D. T.; Ratsch, H. C. (1982) Ozone flux in *Glycine max* (L) Merr: sites of regulation and relationship to leaf injury. Oecologia 53: 179-186.

Taylor, G. E., Jr.; Hanson, P. J.; Baldocchi, D. D. (1988a) Pollutant deposition to individual leaves and plant canopies: sites of regulation and relationship to injury. In: Heck, W. W.; Taylor, O. C.; Tingey, D. T., eds. Assessment of crop loss from air pollutants. New York, NY: Elsevier Applied Science; pp. 227-257.

Taylor, G. E., Jr.; Ross-Todd, B. M.; Gunderson, C. A. (1988b) Action of ozone on foliar gas exchange in *Glycine max* L Merr: a potential role for endogenous stress ethylene. New Phytol. 110: 301-307.
Taylor, G. E.; Owens, J. G.; Grizzard, T.; Selvidge, W. J. (1993) Atmosphere x canopy interactions of nitric acid vapor in loblolly-pine grown in open-top chambers. J. Environ. Qual. 22: 70-80.
Taylor, G. E., Jr.; Johnson, D. W.; Andersen, C. P. (1994) Air pollution and forest ecosystems: a regional to global perspective. Ecol. Appl. 4: 662-689.
Taylor, G.; Ceulemans, R.; Ferris, R.; Gardner, S. D. L.; Shao, B. Y. (2001) Increased leaf area expansion of hybrid poplar in elevated CO_2 from controlled environments to open-top chambers and to FACE. Environ. Pollut. 115: 463-472.
Tegischer, K.; Tausz, M.; Wieser, G.; Grill, D. (2002) Tree-age and needle-age dependent variations of antioxidants and photoprotective pigments in spruce needles at the alpine timberline. Tree Physiol. 22: 591-596.
Temple, P. J. (1988) Injury and growth of Jeffrey pine and giant sequoia in response to ozone and acidic mist. Environ. Exp. Bot. 28: 323-333.
Temple, P. J. (1999) Effects of ozone on understory vegetation in the mixed conifer forest. In: Miller, P. R.; McBride, J. R., eds. Oxidant air pollution impacts in the montane forests of southern California: a case study of the San Bernardino Mountains. New York, NY: Springer; pp. 208-222. (Ecological studies: v. 134).
Temple, P. J.; Riechers, G. H.; Miller, P. R. (1992) Foliar injury responses of ponderosa pine seedlings to ozone, wet and dry acidic deposition, and drought. Environ. Exp. Bot. 32: 101-113.
Temple, P. J.; Riechers, G. H.; Miller, P. R.; Lennox, R. W. (1993) Growth responses of ponderosa pine to long-term exposure to ozone, wet and dry acidic deposition, and drought. Can. J. For. Res. 23: 59-66.
Teramura, A. H.; Sullivan, J. H.; Lyden, J. (1990) Effects of UV-b radiation on soybean yield and seed quality: a 6-year field study. Physiol. Plant. 80: 5-11.
Ter-Mikaelian, M. T.; Korzukhin, M. D. (1997) Biomass equations for sixty-five North American tree species. For. Ecol. Manage. 97: 1-24.
Teskey, R. O.; Dougherty, P. M.; Wiselogel, A. E. (1991) Design and performance of branch chambers suitable for long-term ozone fumigation of foliage in large trees. J. Environ. Qual. 20: 591-595.
Thalmair, M.; Bauw, G.; Thiel, S.; Döhring, T.; Langebartels, C.; Sandermann, H., Jr. (1996) Ozone and ultraviolet B effects on the defense-related proteins β-1,3-glucanase and chitinase in tobacco. J. Plant Physiol. 148: 222-228.
Thom, M.; Maretzki, A. (1985) Evidence for a plasmalemma redox system in sugarcane. Plant Physiol. 77: 873-876.
Tiedemann, A. V.; Firsching, K. H. (1993) Effects of ozone exposure and leaf age of wheat on infection processes of *Septoria nodorum* Berk. Plant Pathol. 42: 287-293.
Tiedemann, A. V.; Firsching, K. H. (2000) Interactive effects of elevated ozone and carbon dioxide on growth and yield of leaf rust-infected versus non-infected wheat. Environ. Pollut. 108: 357-363.
Tiedemann, A. V.; Weigel, H. J.; Jäger, H. J. (1991) Effects of open-top chamber fumigations with ozone on three fungal leaf diseases of wheat and the mycoflora of the phyllosphere. Environ. Pollut. 72: 205-224.
Tingey, D. T.; Blum, U. (1973) Effects of ozone on soybean nodules. J. Environ. Qual. 2: 341-342.
Tingey, D. T.; Taylor, G. E., Jr. (1982) Variation in plant response to ozone: a conceptual model of physiological events. In: Unsworth, M. H.; Ormrod, D. P., eds. Effects of gaseous air pollution in agriculture and horticulture. London, United Kingdom: Butterworth Scientific; pp. 113-138.
Tingey, D. T.; Fites, R. C.; Wickliff, C. (1975) Activity changes in selected enzymes from soybean leaves following ozone exposure. Physiol. Plant. 33: 316-320.
Tingey, D. T.; Standley, C.; Field, R. W. (1976a) Stress ethylene evolution: a measure of ozone effects on plants. Atmos. Environ. 10: 969-974.
Tingey, D. T.; Wilhour, R. G.; Standley, C. (1976b) The effect of chronic ozone exposures on the metabolite content of ponderosa pine seedlings. For. Sci. 22: 234-241.
Tingey, D. T.; Hogsett, W. E.; Lee, E. H.; Herstrom, A. A.; Azevedo, S. H. (1991) An evaluation of various alternative ambient ozone standards based on crop yield loss data. In: Berglund, R. L.; Lawson, D. R.; McKee, D. J. eds. Tropospheric ozone and the environment: papers from an international conference; March 1990; Los Angeles, CA; Pittsburgh, PA: Air & Waste Management Association (A&WMA transactions serie s no. TR-19); pp. 272-288.
Tingey, D. T.; McVeety, B. D.; Waschmann, R.; Johnson, M. G.; Phillips, D. L.; Rygiewicz, P. T.; Olszyk, D. M. (1996) A versatile sun-lit controlled-environment facility for studying plant and soil processes. J. Environ. Qual. 25: 614-625.

Tingey, D. T.; Laurence, J. A.; Weber, J. A.; Greene, J.; Hogsett, W. E.; Brown, S.; Lee, E. H. (2001) Elevated CO_2 and temperature alter the response of *Pinus ponderosa* to ozone: A simulation analysis. Ecol. Appl. 11: 1412-1424.
Tingey, D. T.; Rodecap, K. D.; Lee, E. H.; Hogsett, W. E.; Gregg, J. W. (2002) Pod development increases the ozone sensitivity of *Phaseolus vulgaris*. Water Air Soil Pollut. 139: 325-341.
Tingey, D. T.; Hogsett, W. E.; Lee, E. H.; Laurence, J. A. (2004) Stricter ozone ambient air quality standard has beneficial effect on Ponderosa pine in California. Environ. Manage. 34: 397-405.
Tissue, D. T.; Griffin, K. L.; Ball, T. (1999) Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO_2. Tree Physiol. 19: 221-228.
Tjoelker, M. G.; Luxmoore, R. J. (1991) Soil nitrogen and chronic ozone stress influence physiology, growth and nutrient status of *Pinus taeda* L. and *Liriodendron tulipifera* L. seedlings. New Phytol. 119: 69-81.
Tjoelker, M. G.; Volin, J. C.; Oleksyn, J.; Reich, P. B. (1993) Light environment alters response to ozone stress in seedlings of *Acer saccharum* Marsh and hybrid *Populus* L. I. *In situ* net photosynthesis, dark respiration and growth. New Phytol. 124: 627-636.
Tjoelker, M. G.; Volin, J. C.; Oleksyn, J.; Reich, P. B. (1994) An open-air system for exposing forest-canopy branches to ozone pollution. Plant Cell Environ. 17: 211-218.
Tjoelker, M. G.; Volin, J. C.; Oleksyn, J.; Reich, P. B. (1995) Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment. Plant Cell Environ. 18: 895-905.
Tognetti, R.; Cherubini, P.; Innes, J. L. (2000) Comparative stem-growth rates of Mediterranean trees under background and naturally enhanced ambient CO_2 levels. New Phytol. 146: 59-74.
Tokarska-Schlattner, M.; Fink, A.; Castillo, F. J.; Crespi, P.; Crevecoeur, M.; Greppin, H.; Tacchini, P. (1997) Effects of ozone on the plasma membrane proteins in *Arabidopsis thaliana* (L.) leaves. Plant Cell Environ. 20: 1205-1211.
Tomlinson, H.; Rich, S. (1968) The ozone resistance of leaves as related to their sulfhydryl and adenosine triphosphate content. Phytopathology 58: 808-810.
Tomlinson, H.; Rich, S. (1971) Effect of ozone on sterols and sterol derivatives in bean leaves. Phytopathology 61: 1404-1405.
Tonneijck, A. E. G. (1994) Effects of various ozone exposures on the susceptibility of bean leaves (*Phaseolus vulgaris* L) to *Botrytis cinerea*. Environ. Pollut. 85: 59-65.
Tonneijck, A. E. G.; Leone, G. (1993) Changes in susceptibility of bean leaves (*Phaseolus vulgaris*) to *Sclerotinia sclerotiorum* and *Botrytis cinerea* by pre-inoculative ozone exposures. Neth. J. Plant Pathol. 99: 313-322.
Tonneijck, A. E. G.; Van Dijk, C. J. (1994) Combined effects of ozone and ammonia on growth and yield of bean (*Phaseolus vulgaris* L cv Pros). In: Fuhrer, J.; Achermann, B. eds. Critical levels for ozone: a UN-ECE workshop report; November 1993; Bern, Switzerland. Liebefeld-Bern, Switzerland: Swiss Federal Research Station for Agricultural Chemistry and Environmental Hygiene; pp. 282-286 (Schriftenreihe der FAC Liebefeld no. 16).
Tonneijck, A. E. G.; Van Dijk, C. J. (1997) Effects of ambient ozone on injury and yield of *Phaseolus vulgaris* at four rural sites in The Netherlands as assessed by using ethylenediurea (EDU). New Phytol. 135: 93-100.
Tonneijck, A. E. G.; Van Dijk, C. J. (1998) Responses of bean (*Phaseolus vulgaris* L cv Pros) to chronic ozone exposure at two levels of atmospheric ammonia. Environ. Pollut. 99: 45-51.
Tonneijck, A. E. G.; Van Dijk, C. J. (2002a) Assessing effects of ambient ozone on injury and yield of bean with ethylenediurea (EDU): three years of plant monitoring at four sites in The Netherlands. Environ. Monit. Assess. 77: 1-10.
Tonneijck, A. E. G.; Van Dijk, C. J. (2002b) Injury and growth response of subterranean clover to ambient ozone as assessed by using ethylenediurea (EDU): Three years of plant monitoring at four sites in The Netherlands. Environ. Exp. Bot. 48: 33-41.
Topa, M. A.; Vanderklein, D. W.; Corbin, A. (2001) Effects of elevated ozone and low light on diurnal and seasonal carbon gain in sugar maple. Plant Cell Environ. 24: 663-677.
Torsethaugen, G.; Pitcher, L. H.; Zilinskas, B. A.; Pell, E. J. (1997) Overproduction of ascorbate peroxidase in the tobacco chloroplast does not provide protection against ozone. Plant Physiol. 114: 529-537.
Treshow, M.; Stewart, D. (1973) Ozone sensitivity of plants in natural communities. Biol. Conserv. 5: 209-214.
Trevathan, L. E.; Moore, L. D.; Orcutt, D. M. (1979) Symptom expression and free sterol and fatty acid composition of flue-cured tobacco plants exposed to ozone. Phytopathology 69: 582-585.
Tuovinen, J.-P.; Ashmore, M. R.; Emberson, L. D.; Simpson, D. (2004) Testing and improving the EMEP ozone deposition module. Atmos. Environ. 38: 2372-2385.
Turcsányi, E.; Lyons, T.; Plöchl, M.; Barnes, J. (2000) Does ascorbate in the mesophyll cell walls form the first line of defence against ozone? Testing the concept using broad bean (*Vicia faba* L.). J. Exp. Bot. 51: 901-910.

U.S. Congress (2002) Food security and rural investment act of 2002, PL 107-171, May 13, 2002. Washington, DC: U.S. Government Printing Office.

U.S. Environmental Protection Agency. (1978) Air quality criteria for ozone and other photochemical oxidants. Research Triangle Park, NC: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office; report no. EPA-600/8-78-004. Available from: NTIS, Springfield, VA; PB80-124753.

U.S. Environmental Protection Agency. (1986) Air quality criteria for ozone and other photochemical oxidants. Research Triangle Park, NC: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office; report nos. EPA-600/8-84-020aF-eF. 5v. Available from: NTIS, Springfield, VA; PB87-142949.

U.S. Environmental Protection Agency. (1992) Summary of selected new information on effects of ozone on health and vegetation: supplement to 1986 air quality criteria for ozone and other photochemical oxidants. Research Triangle Park, NC: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office; report no. EPA/600/8-88/105F. Available from: NTIS, Springfield, VA; PB92-235670.

U.S. Environmental Protection Agency. (1996a) Review of national ambient air quality standards for ozone: assessment of scientific and technical information OAQPS staff paper. Research Triangle Park, NC: Office of Air Quality Planning and Standards; report no. EPA/452/R-96/007. Available from: NTIS, Springfield, VA; PB96-203435/XAB. Available: http://www.epa.gov/ttn/naaqs/standards/ozone/s_O_3_pr_sp.html (9 September 2003).

U.S. Environmental Protection Agency. (1996b) Air quality criteria for ozone and related photochemical oxidants. Research Triangle Park, NC: Office of Research and Development; report nos. EPA/600/AP-93/004aF-cF. 3v. Available from: NTIS, Springfield, VA; PB96-185582, PB96-185590, and PB96-185608. Available: www.epa.gov/ncea/ozone.htm.

U.S. National Park Service (2003) Ozone sensitive plant species on National Park Service and U.S. Fish and Wildlife Service lands: results of a June 24-25, 2003 workshop, Baltimore, Maryland. Denver, CO: U.S. Department of the Interior, National Park Service Air Resources Division, U.S. Fish and Wildlife Service Air Quality Branch; Natural Resource Report NPS/NRARD/NRR-2003/01. Available at www2.nature.nps.gov/ard/pubs/index.htm.

United Nations Economic Commission for Europe (UNECE). (1988) ECE Critical Levels Workshop; Bad Harzburg, Germany [final report]. Geneva, Switzerland: United Nations Economic Commission for Europe.

United Nations Environment Programme (UNEP) (1993) Environmental data report 1993-94. 4th ed. Oxford, United Kingdom: Blackwell Publishers.

United Nations Environment Programme (UNEP) (1999) Geo-2000: global environmental outlook 2000. London, United Kingdom: Earthscan Publishers; Available: http://www.unep.org/geo2000/english/index.htm [26 July 2002].

Unsworth, M. H.; Hogsett, W. E. (1996) Combined effects of changing CO_2, temperature, UV-B radiation and O_3 on crop growth. In: Bazzaz, F.; Sombroek, W., eds. Global climate change and agricultural production. John Wiley & Sons; pp. 171-197.

Unsworth, M. H.; Heagle, A. S.; Heck, W. W. (1984a) Gas exchange in open-top field chambers - I measurement and analysis of atmospheric resistances to gas exchange. Atmos. Environ. 18: 373-380.

Unsworth, M. H.; Heagle, A. S.; Heck, W. W. (1984b) Gas exchange in open-top field chambers - II resistances to ozone uptake by soybeans. Atmos. Environ. 18: 381-385.

Utriainen, J.; Holopainen, T. (2001) Nitrogen availability modifies the ozone responses of Scots pine seedlings exposed in an open-field system. Tree Physiol. 21: 1205-1213.

Utriainen, J.; Janhunen, S.; Helmisaari, H. S.; Holopainen, T. (2000) Biomass allocation, needle structural characteristics and nutrient composition in Scots pine seedlings exposed to elevated CO_2 and O_3 concentrations. Trees (Berlin, Ger.) 14: 475-484.

Vacek, S.; Bastl, M.; Leps, J. (1999) Vegetation changes in forests of the Krkonose Mountains over a period of air pollution stress (1980-1995). Plant Ecol. 143: 1-11.

Vahala, J.; Schlagnhaufer, C. D.; Pell, E. J. (1998) Induction of an ACC synthase cDNA by ozone in light-grown *Arabidopsis thaliana* leaves. Physiol. Plant. 103: 45-50.

Vahala, J.; Keinänen, M.; Schützendübel, A.; Polle, A.; Kangasjärvi, J. (2003a) Differential effects of elevated ozone on two hybrid aspen genotypes predisposed to chronic ozone fumigation Role of ethylene and salicylic acid. Plant Physiol. 132: 196-205.

Vahala, J.; Ruonala, R.; Keinanen, M.; Tuominen, H.; Kangasjarvi, J. (2003b) Ethylene insensitivity modulates ozone-induced cell death in birch. Plant Physiol. 132: 185-195.

Van Camp, W.; Willekens, H.; Bowler, C.; Van Montagu, M.; Inzé, D.; Reupold-Popp, P.; Sandermann, H., Jr.; Langebartels, C. (1994) Elevated levels of superoxide dismutase protect transgenic plants against ozone damage. Biotechnology 12: 165-168.

Van De Staaij, J. W. M.; Lenssen, G. M.; Stroetenga, M.; Rozema, J. (1993) The combined effects of elevated CO_2 levels and UV-B radiation on growth characteristics of *Elymus athericus* (= *E. pycnanthus*). Vegetatio 104/105: 433-439.

Van De Staaij, J. W. M.; Tonneijck, A. E. G.; Rozema, J. (1997) The effect of reciprocal treatments with ozone and ultraviolet-B radiation on photosynthesis and growth of perennial grass *Elymus athericus*. Environ. Pollut. 97: 281-286.

Van Den Driessche, R. (1978) Seasonal changes in root growth capacity and carbohydrates in red pine and white spruce nursery seedlings. In: Riedacker, A.; Gagnaire-Michard, J., eds. Root physiology and symbiosis: proceedings of the IUFRO symposium; September; Nancy, France. Washington, DC: International Union of Forestry Research Organizations; pp. 6-19.

Van Den Driessche, R. (1991) New root growth of Douglas-fir seedlings at low carbon dioxide concentration. Tree Physiol. 8: 289-295.

Van Den Driessche, R.; Langebartels, C. (1994) Foliar symptoms, ethylene biosynthesis and water use of young Norway spruce (*Picea abies* (L) Karst) exposed to drought and ozone. Water Air Soil Pollut. 78: 153-168.

Van Oijen, M.; Ewart, F. (1999) The effects of climatic variation in Europe on the yield response of spring wheat cv Minaret to elevated CO_2 and O_3: an analysis of open-top chamber experiments by means of two crop growth simulation models. Eur. J. Agron. 10: 249-264.

Van Oijen, M.; Schapendonk, A. H. C. M.; Jansen, M. J. H.; Pot, C. S.; Maciorowski, R. (1999) Do open-top chambers overestimate the effects of rising CO_2 on plants? An analysis using spring wheat. Global Change Biol. 5: 411-421.

Vanacker, H.; Carver, T. L. W.; Foyer, C. H. (2000) Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during the hyper-sensitive response in the barley-powdery mildew interaction. Plant Physiol. 123: 1289-1300.

VanderHeyden, D.; Skelly, J.; Innes, J.; Hug, C.; Zhang, J.; Landolt, W.; Bleuler, P. (2001) Ozone exposure thresholds and foliar injury on forest plants in Switzerland. Environ. Pollut. 111: 321-331.

Vandermeiren, K.; De Temmerman, L.; Hookham, N. (1995) Ozone sensitivity of *Phaseolus vulgaris* in cultivar differences, growth stage and growing conditions. Water Air Soil Pollut. 85: 1455-1460.

Vandermeiren, K.; Black, C.; Lawson, T.; Casanova, M. A.; Ojanperä, K. (2002) Photosynthetic and stomatal responses of potatoes grown under elevated CO_2 and/or O_3–results from the European CHIP-programme. Eur. J. Agron. 17: 337-352.

Vann, D. R.; Strimbeck, G. R.; Johnson, A. H. (1995) Effects of mist acidity and ambient ozone removal on montane red spruce. Tree Physiol. 15: 639-647.

Volin, J. C.; Reich, P. B.; Givnish, T. J. (1998) Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: species respond similarly regardless of photosynthetic pathway or plant functional group. New Phytol. 138: 315-325.

Volk, M.; Geissmann, M.; Blatter, A.; Contat, F.; Fuhrer, J. (2003) Design and performance of a free-air exposure system to study long-term effects of ozone on grasslands. Atmos. Environ. 37: 1341-1350.

Vollenweider, P.; Woodcock, H.; Kelty, M. J.; Hofer, R.-M. (2003a) Reduction of stem growth and site dependency of leaf injury in Massachusetts black cherries exhibiting ozone symptoms. Environ. Pollut. 125: 467-480.

Vollenweider, P.; Ottiger, M.; Günthardt-Goerg, M. S. (2003b) Validation of leaf ozone symptoms in natural vegetation using microscopical methods. Environ. Pollut. 124: 101-118.

Von Gunten, U. (2003) Ozonation of drinking water: part I Oxidation kinetics and product formation. Water Res. 37: 1443-1467.

Vorne, V.; Ojanperä, K.; De Temmerman, L.; Bindi, M.; Högy, P.; Jones, M. B.; Lawson, T.; Persson, K. (2002) Effects of elevated carbon dioxide and ozone on potato tuber quality in the European multiple-site experiment 'CHIP-project'. Eur. J. Agron. 17: 369-381.

Vozzo, S. F.; Miller, J. E.; Pursley, W. A.; Heagle, A. S. (1995) Effects of ozone and water deficit on field-grown soybean: I. Leaf gas exchange. J. Environ. Qual. 24: 663-670.

Waite, C. E.; DeHayes, D. H.; Rebbeck, J.; Schier, G. A.; Johnson, A. H. (1994) The influence of elevated ozone on freezing tolerance of red spruce seedlings. New Phytol. 126: 327-335.

Walcek, C. J.; Yuan, H. H.; Stockwell, W. R. (1997) The influence of aqueous-phase chemical reactions on ozone formation in polluted and nonpolluted clouds. Atmos. Environ. 31: 1221-1237.

Wallin, G.; Skärby, L.; Selldén, G. (1992) Long-term exposure of Norway spruce, *Picea abies* (L.) Karst, to ozone in open-top chambers 3 Effects on the light response of net photosynthesis in shoots of different ages. New Phytol. 121: 387-394.

Wallin, G.; Karlsson, P. E.; Selldén, G.; Ottosson, S.; Medin, E.-L.; Pleijel, H.; Skärby, L. (2002) Impact of four years exposure to different levels of ozone, phosphorus and drought on chlorophyll, mineral nutrients, and stem volume of Norway spruce, *Picea abies*. Physiol. Plant. 114: 192-206.

Ward, E. R.; Uknes, S. J.; Williams, S. C.; Dincher, S. S.; Wiederhold, D. L.; Alexander, D. C.; Ahi-Goy, P.; Métraux, J. P.; Ryals, J. A. (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell 3: 1084-1094.

Warwick, K. R.; Taylor, G. (1995) Contrasting effects of tropospheric ozone on five native herbs which coexist in calcareous grassland. Global Change Biol. 1: 143-151.

Weber, J.; Grulke, N. E. (1995) Effects of air pollution on stem growth. In: Gartner, B., ed. Stems and trunks in plant form and function. San Diego, CA: Academic Press; pp. 343-364.

Weber, J. A.; Clark, C. S.; Hogsett, W. E. (1993) Analysis of the relationships among O_3 uptake, conductance, and photosynthesis in needles of *Pinus ponderosa*. Tree Physiol. 13: 157-172.

Weiner, J.; Fredro-Boniecki, S.; Reed, D.; Maclean, A.; Strong, M. (1997) NiepoLomice forest-A GIS analysis of ecosystem response to industrial pollution. Environ. Pollut. 3: 381-388.

Weinstein, D. A.; Yanai, R. D. (1994) Integrating the effects of simultaneous multiple stresses on plants using the simulation model TREGRO. J. Environ. Qual. 23: 418-428.

Weinstein, D. A.; Samuelson, L. J.; Arthur, M. A. (1998) Comparison of the response of red oak (*Quercus rubra*) seedlings and mature trees to ozone exposure using simulation modeling. Environ. Pollut. 102: 307-320.

Weinstein, D. A.; Gollands, B.; Retzlaff, W. A. (2001) The effects of ozone on a lower slope forest of the Great Smoky Mountain National Park: simulations linking an individual tree model to a stand model. For. Sci. 47: 29-42.

Weinstein, D. A.; Woodbury, P. B.; Gollands, B.; King, P.; Lepak, L.; Pendleton, D. (2002) Assessment of effects of ozone on forest resources in the southern Appalachian mountains. Asheville, NC: Southern Appalachian Mountains Initiative. Available: http://cycas.cornell.edu/ebp/pmg.html [19 March, 2003].

Welfare, K.; Flowers, T. J.; Taylor, G.; Yeo, A. R. (1996) Additive and antagonistic effects of ozone and salinity on the growth, ion contents and gas exchange of five varieties of rice (*Oryza sativa* L). Environ. Pollut. 92: 257-266.

Wellburn, A. R. (1990) Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilizers? New Phytol. 115: 395-429.

Wellburn, A. (1994) Air pollution and climate change: the biological impact. Harlow, Essex, United Kingdom: Longman Scientific & Technical.

Wellburn, F. A. M.; Wellburn, A. R. (1994) Atmospheric ozone affects carbohydrate allocation and winter hardiness of *Pinus halepensis* (Mill). J. Exp. Bot. 274: 607-614.

Wellburn, F. A. M.; Wellburn, A. R. (1996) Variable patterns of antioxidant protection but similar ethene emission differences in several ozone-sensitive and ozone-tolerant plant selections. Plant Cell Environ. 19: 754-760.

Werner, H.; Fabian, P. (2002) Free-air fumigation of mature trees A novel system for controlled ozone enrichment in grown-up beech and spruce canopies. Environ. Sci. Pollut. Res. 9: 117-121.

Wesely, M. L. (1989) Parameterization of surface resistances to gaseous dry deposition in regional-scale numerical models. Atmos. Environ. 23: 1293-1304.

Wesely, M. L.; Hicks, B. B. (2000) A review of the current status of knowledge on dry deposition. Atmos. Environ. 34: 2261-2282.

Westenbarger, A. D.; Frisvold, G. B. (1994) Agricultural exposure to ozone and acid precipitation. Atmos. Environ. 28: 2895-2907.

Westman, W. E. (1979) Oxidant effects on Californian coastal sage scrub. Science (Washington, DC) 205: 1001-1003.

Westman, W. E. (1981) Factors influencing the distribution of species of Californian coastal sage scrub. Ecology 62: 439-455.

Weubbles, D. J.; Jain, A.; Edmonds, J.; Harvey, D.; Hayhoe, K. (1999) Global change: state of the science. Environ. Pollut. 100: 57-86.

Wezyk, P.; Guzik, M. (2002) The use of "photogrammetry-GIS" (P-GIS) for the analysis of changes in the Tatra Mountains' natural environment. In: Widacki, W.; Bytnerowicz, A.; Riebau, A., eds. A Message from the Tatra – geographical information systems and remote sensing in mountain environmental research. Krakow, Poland: Jagiellonian University Press.

Wheeler, T. R.; Hong, T. D.; Ellis, R. H.; Batts, G. R.; Morrison, J. I. L.; Hadley, P. (1996) The duration and rate of grain growth, and harvest index, of wheat (*Triticum aestivum* L) in response to temperature and CO_2. J. Exper. Bot. 47: 623-630.

Whitfield, C. P.; Davison, A. W.; Ashenden, T. W. (1996a) Interactive effects of ozone and soil volume on *Plantago major*. New Phytol. 134: 287-294.

Whitfield, R. G.; Biller, W. F.; Jusko, M. J.; Keisler, J. M. (1996b) A probabilistic assessment of health risks associated with short-term exposure to tropospheric ozone. Argonne, IL: Argonne National Laboratory, Decision and Information Sciences Division; report no. ANL/DIS-3.

Whitfield, C. P.; Davison, A. W.; Ashenden, T. W. (1997) Artificial selection and heritability of ozone resistance in two populations of *Plantago major*. New Phytol. 137: 645-655.

Whitfield, C. P.; Davison, A. W.; Ashenden, T. W. (1998) The effects of nutrient limitation on the response of *Plantago major* to ozone. New Phytol. 140: 219-230.

Whittaker, J. B. (1994) Interactions between insects and air pollutants. In: Alscher, R. G.; Wellburn, A. R., eds. Plant responses to the gaseous environment. London, United Kingdom: Chapman and Hall; pp. 365-384.

Wickman, B. E. (1992) Forest Health in the Blue Mountains: the influence of insects and diseases. Portland OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station; general technical report PNW-GTR-295. (Quigley, T. M., ed. Forest health in the Blue Mountains: science perspectives).

Widacki, W.; Bytnerowicz, A.; Riebau, A. (2002) A message from the Tatra – geographical information systems and remote sensing in mountain environmental reseach. Krakow, Poland: Jagiellonian University Press.

Wieser, G. (1997) Ozone impact on photosynthetic capacity of mature and young Norway spruce (*Picea abies* (L) Karst): External versus internal exposure. Phyton (Austria) 37: 297-302.

Wieser, G.; Emberson, L. (2004) Evaluation of the stomatal and conductance formulation in the EMEP ozone deposition model for *Picea abies*. Atmos. Environ. 38: 2339-2348.

Wieser, G.; Havranek, W. M. (1993) Ozone uptake in the sun and shade crown of spruce: quantifying the physiological effects of ozone exposure. Trees (Berlin, Ger.) 7: 227-232.

Wieser, G.; Havranek, W. M. (1995) Environmental control of ozone uptake in *Larix decidua* Mill: a comparison between different altitudes. Tree Physiol. 15: 253-258.

Wieser, G.; Häsler, R.; Götz, B.; Koch, W.; Havranek, W. M. (1999) Seasonal ozone uptake of mature evergreen conifers at different altitudes. Phyton (Austria) 39: 233-240.

Wieser, G.; Tegischer, K.; Tausz, M.; Häberle, K. H.; Grams, T. E. E.; Matyssek, R. (2002) Age effects on Norway spruce (*Picea abies*) susceptibility to ozone uptake: a novel approach relating stress avoidance to defense. Tree Physiol. 22: 583-590.

Wilbourn, S.; Davison, A. W.; Ollerenshaw, J. H. (1995) The use of an unenclosed field fumigation system to determine the effects of elevated ozone on a grass-clover mixture. New Phytol. 129: 23-32.

Willekens, H.; Van Camp, M.; Van Montagu, M.; Inze, D.; Langebartels, C.; Sandermann, H., Jr. (1994) Ozone, sulfur dioxide and ultraviolet B have similar effects on mRNA accumulation of fantioxidant genes in *Nicotiana plumbaginifolia* L. Plant Physiol. 106: 1007-1014.

Williams, T. P. W. (1994) Use of passive monitors to assess plant bioindicators for ground-level ozone [master's thesis]. University of New Brunswick; 131 pp.

Williams, J.; Bulman, M. P.; Neill, S. J. (1994) Wilt-induced aba biosynthesis, gene expression and down regulation of *rbcS* mRNA levels in *Arabidopsis thaliana*. Physiol. Plant. 91: 177-182.

Wiltshire, J. J. J.; Wright, C. J.; Unsworth, M. H. (1992) A new method for exposing mature trees to ozone episodes. For. Ecol. Manage. 51: 115-120.

Wiltshire, J. J. J.; Unsworth, M. H.; Wright, C. J. (1994) Seasonal changes in water use of ash trees exposed to ozone episodes. New Phytol. 127: 349-354.

Wiltshire, J. J. J.; Wright, C. J.; Colls, J. J.; Craigon, J.; Unsworth, M. H. (1996) Some foliar characteristics of ash trees (*Fraxinus exelsior*) exposed to ozone episodes. New Phytol. 134: 623-630.

Winner, W. E.; Lefohn, A. S.; Cotter, I. S.; Greitner, C. S.; Nellessen, J.; McEvoy, L. R., Jr.; Olson, R. L.; Atkinson, C. J.; Moore, L. D. (1989) Plant responses to elevational gradients of O_3 exposures in Virginia. Proc. Natl. Acad. Sci. U. S. A. 86: 8828-8832.

Winner, W. E.; Coleman, J. S.; Gillespie, C.; Mooney, H. A.; Pell, E. J. (1991) Consequences of evolving reistance to air pollutants. In: Taylor, G. E.; Pitelka, L. F.; Clegg, M. T., eds. Ecological genetics and air pollution. New York, NY: Springer-Verlag; pp. 177-202.

Wolf, J.; Van Oijen, M. (2003) Model simulation of effects of changes in climate and atmospheric CO_2 and O_3 on tuber yield potential of potato (cv Bintje) in the European Union. Agric. Ecosyst. Environ. 94: 141-157.

Wolff, K.; Morgan-Richards, M.; Davison, A. W. (2000) Patterns of molecular genetic variation in *Plantago major* and *P. intermedia* in relation to ozone resistance. New Phytol. 145: 501-509.
Wöllmer, H.; Kottke, I. (1990) Fine root studies *in situ* and in the laboratory. Environ. Pollut. 68: 383-407.
Wolters, V. (1998) Functional aspects of animal diversity in soil: introduction and overview. Appl. Soil Ecol. 10: 185-190.
Wonisch, A.; Müller, M.; Tausz, M.; Soja, G.; Grill, D. (1998) Stress-physiological investigations and chromosomal analyses on cloned Norway spruce trees exposed to various levels of ozone in open-top chambers. Chemosphere 36: 709-714.
Wonisch, A.; Müller, M.; Tausz, M.; Soja, G.; Grill, D. (1999) Simultaneous analyses of chromosomes in root meristems and of the biochemical status of needle tissues of three different clones of Norway spruce trees challenged with moderate ozone levels. Eur. J. For. Pathol. 29: 281-294.
Woodbury, P. B.; Laurence, J. A.; Hudler, G. W. (1994) Chronic ozone exposure increases the susceptibility of hybrid *Populus* to disease caused by *Septoria musiva*. Environ. Pollut. 86: 109-114.
Wu, Y. X.; Tiedemann, A. V. (2002) Impact of fungicides on active oxygen species and antioxidant enzymes in spring barley (*Hordeum vulgare* L.) exposed to ozone. Environ. Pollut. 116: 37-47.
Wulff, A.; Hänninen, O.; Tuomainen, A.; Kärenlampi, L. (1992) A method for open-air exposure of plants to ozone. Ann. Bot. Fennici 29: 253-262.
Wullschleger, S. D.; Hanson, P. J.; Edwards, G. S. (1996) Growth and maintenance respiration in leaves of northern red oak seedlings and mature trees after 3 years of ozone exposure. Plant Cell Environ. 19: 577-584.
Wustman, B. A.; Oksanen, E.; Karnosky, D. F.; Noormets, A.; Isebrands, J. G.; Pregitzer, K. S.; Hendrey, G. R.; Sober, J.; Podila, G. K. (2001) Effects of elevated CO_2 and O_3 on aspen clones varying in O_3 sensitivity: can CO_2 ameliorate the harmful effects of O_3? Environ. Pollut. 115: 473-481.
Yalpani, N.; Enyedi, A. J.; León, J.; Raskin, I. (1994) Ultraviolet light and ozone stimulate accumulation of salicyclic acid, pathogenesis-related proteins and virus resistance in tobacco. Planta 193: 372-376.
Yang, S. F.; Hoffman, N. E. (1984) Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35: 155-189.
Yonekura, T.; Dokiya, Y.; Fukami, M.; Izuta, T. (2001) Effects of ozone and/or soil water stress on growth and photosynthesis of *Fagaus crenata* seedlings. Water Air Soil Pollut. 130: 965-970.
Yoshida, L. C.; Gamon, J. A.; Andersen, C. P. (2001) Differences in above- and below-ground responses to ozone between two populations of a perennial grass. Plant Soil 233: 203-211.
Young, T. F.; Sanzone, S., eds. (2002) A framework for assessing and reporting on ecological condition: an SAB report. Washington, DC: U.S. Environmental Protection Agency, Science Advisory Board; report no. EPA-SAB-EPEC-02-009. Available: http://www.epa.gov/sab/pdf/epec02009.pdf [9 December, 2003].
Younglove, T.; McCool, P. M.; Musselman, R. C.; KahI, M. E. (1994) Growth-stage dependent crop yield response to ozone exposure. Environ. Pollut. 86: 287-295.
Yun, S. C.; Laurence, J. A. (1999a) The response of sensitive and tolerant clones of *Populus tremuloides* to dynamic ozone exposure under controlled environmental conditions. New Phytol. 143: 305-313.
Yun, S. C.; Laurence, J. A. (1999b) The response of clones of *Populus tremuloides* differing in sensitivity to ozone in the field. New Phytol. 141: 411-421.
Yunus, M.; Iqbal, M. e. (1996) Plant response to air pollution. Chichester, United Kingdom: John Wiley & Sons Ltd.
Yuska, D. E.; Skelly, J. M.; Ferdinand, J. A.; Stevenson, R. E.; Savage, J. E.; Mulki, J.; Hines, A. (2003) Use of bioindicators and passive sampling devices to evaluate ambient ozone concentrations in north central Pennsylvania. Environ. Pollut. 125: 71-80.
Zambrano, A.; Nash, T. H., III. (2000) Lichen responses to short-term transplantation in Desierto de los Leones, Mexico City. Environ. Pollut. 107: 407-412.
Zeller, K.; Nikolov, N. (2000) Quantifying simultaneous fluxes of ozone, carbon dioxide, and water vapor above a subalpine forest ecosystem. Environ. Pollut. 107: 20.
Zhang, X.; Zhang, L.; Dong, F.; Gao, J.; Galbraith, D. W.; Song, C. P. (2001) Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba*. Plant Physiol. 126: 1438-1448.
Zinser, C.; Jungblut, T. H.; Seidlitz, H. K.; Schnitzler, J. P.; Ernst, D.; Sandermann, H., Jr. (2000) The effect of ozone in Scots pine (*Pinus sylvestris* L): gene expression, biochemical changes and interactions with UV-B radiation. Plant Cell Environ. 23: 975-982.
Ziska, L.; Bunce, J. A. (1997) The role of temperature in determining the stimulation of CO_2 assimilation at elevated carbon dioxide concentrations in soybean seedlings. Physiol. Plant. 100: 126-132.

EPA

United States
Environmental Protection
Agency

Please make all necessary changes in the below label, detach copy or copy, and return to the address in the upper left-hand corner.

If you do not wish to receive these reports CHECK HERE ☐; detach copy or copy, and return to the address in the upper left-hand corner.

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA
PERMIT No. G-35

National Center for
Environmental Assessment
Research Triangle Park, NC 27711

Official Business
Penalty for Private Use
$300

EPA 600/R-05/004cF
February 2006