EPA

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

Volume III of III

Air Quality Criteria for Ozone and Related Photochemical Oxidants

Volume III

National Center for Environmental Assessment-RTP Office
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PREFACE

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

In 1971, the U.S. Environmental Protection Agency (EPA) promulgated National Ambient Air Quality Standards (NAAQS) to protect the public health and welfare from adverse effects of photochemical oxidants. The EPA promulgates the NAAQS on the basis of scientific information contained in air quality criteria issued under Section 108 of the Clean Air Act. Following the review of criteria as contained in the EPA document, Air Quality Criteria for Ozone and other Photochemical Oxidants published in 1978, the chemical designation of the standards was changed from photochemical oxidants to ozone (O_3) in 1979 and a 1-hour O_3 NAAQS was set. The 1978 document focused primarily on the scientific air quality criteria for O_3 and, to a lesser extent, on those for other photochemical oxidants such as hydrogen peroxide and the peroxyacyl nitrates, as have subsequent revised versions of the ozone document.

To meet Clean Air Act requirements noted above for periodic review of criteria and NAAQS, the O_3 criteria document, *Air Quality Criteria for Ozone and Other Photochemical*

Oxidants, was next revised and then released in August 1986; and a supplement, *Summary of Selected New Information on Effects of Ozone on Health and Vegetation*, was issued in January 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O_3 was not appropriate at that time. That decision, however, did not take into account some of the newer scientific data that became 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, which was completed in 1996 and provided scientific bases supporting the setting by EPA in 1997 of an 8-h O_3 NAAQS that is currently in force together with the 1-h O_3 standard.

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 Ozone Air Quality Criteria Document (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, some other scientific data are also presented and evaluated in order to provide a better understanding of the nature, sources, distribution, measurement, and concentrations of O_3 and related photochemical oxidants and their precursors in the environment. The document assesses pertinent literature available through 2004.

The present draft document (dated January 2005) is being released for public comment and review by the Clean Air Scientific Advisory Committee (CASAC) to obtain comments on the organization and structure of the document, the issues addressed, the approaches employed in assessing and interpreting the newly available information on O_3 exposures and effects, and the key findings and conclusions arrived at as a consequence of this assessment. Public comments and recommendations will be taken into account making any appropriate further revisions to this document for incorporation into a Second External Review Draft. That draft will be released for further public comment and CASAC review before last revisions are made in response and incorporated into a final version to be completed by early 2006. Evaluations contained in the present document will be drawn on to provide inputs to associated PM Staff Paper analyses prepared by EPA's Office of Air Quality Planning and Standards (OAQPS) to pose options for consideration by the EPA Administrator with regard to proposal and, ultimately, promulgation of decisions on potential retention or revision, as appropriate, of the current 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. The document also evaluates ambient O_3 environmental effects on vegetation and ecosystems, man-made materials, and surface level solar UV radiation flux and global climate change, to support decision making on secondary O_3 NAAQS.

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Air Quality Criteria for Ozone and Related Photochemical Oxidants
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VOLUME I

**Air Quality Criteria for Ozone and Related
Photochemical Oxidants
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Abbreviations and Acronyms

AQCD	Air Quality Criteria Document
$C \times T$	Concentration times duration of exposure
CI	color index
CO_2	carbon dioxide
COP	Conference of Parties
CTM	Chemistry Transport Model
DNA	Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
GSH	Glutathione
H^+	hydrogen ion
H_2O_2	hydrogen peroxide
HFC	hydrofluorocarbon
HO	hydroxyl
HO_2	hydroperoxyl; hydroperoxy
IPCC	Intergovernmental Panel on Climate Change
IR	infrared radiation
mRNA	Messenger ribonucleic acid
NHAPS	National Human Activity Pattern Survey
NMHC	nonmethane hydrocarbon
NO_2	nitrogen dioxide
NO_x	nitrogen oxides
NRC	National Research Council
$O(^1D)$	electronically excited oxygen atom
O_3	ozone
OH	hydroxyl; hydroxy
PAN	Peroxyacetyl nitrate

RNA	Ribonucleic acid
RO$_2$	organic peroxyl; organic peroxy
SO$_2$	sulfur dioxide
SOD	Superoxide dismutase
SUM00	Sum of all hourly average concentrations
SUM06	Seasonal sum of all hourly average concentrations ≥ 0.06 ppm
TAR	Third Assessment Report
UNEP	United Nations Environment Program
UV	Ultraviolet
UV-A	
UV-B	Ultraviolet radiation of wavelengths from 280 to 320 nm
UV-C	
W126	cumulative integrated exposure index with a sigmoidal weighting function
WMO	World Meteorological Organization
ZAPS	Zonal Air Pollution System

9. ENVIRONMENTAL EFFECTS: OZONE EFFECTS ON VEGETATION AND ECOSYSTEMS

9.1 INTRODUCTION

The preceding chapters of this document focused on discussion of: (a) background information regarding ozone (O_3)-related atmospheric chemistry, air quality and exposure aspects; and (b) dosimetric/health effects aspects, as well as the integrative synthesis of key information drawn from such chapters of most importance for EPA review of the primary O_3 NAAQS. On the other hand, this and the next two chapters assess available scientific information on O_3-related welfare effects of most pertinence for the review of secondary O_3 NAAQS. This includes discussion of three classes of environmental effects:

- O_3 effects on vegetation and ecosystem (Chapter 9);

- the role of tropospheric O_3 in climate change, including determining of ground-level UV-B flux (Chapter 10); and

- O_3 effects on manmade materials (Chapter 11).

As for the organizational structure of this chapter, after this opening Introduction, Section 9.2 highlights key features of methodologies used in vegetation research, followed by discussion of vegetation species response/mode-of-action aspects (Section 9.3) and factors that modify functional and growth responses (Section 9.4). Effects-based air quality exposure and dose-response indices are next discussed in Section 9.5, followed by assessment of O_3 exposure-plant response relationships (Section 9.6) and effects on natural ecosystems (Section 9.7). The economic evaluation of O_3 effects on agriculture, forestry, and natural ecosystems is then discussed in Section 9.8. Lastly, the chapter closes with Section 9.9, an overall summary of key findings and conclusions.

9.2   METHODOLOGIES USED IN VEGETATION RESEARCH

9.2.1 Introduction

The scale of investigations into the direct effects of O_3 on plants range from evaluation of sub-cellular to cellular, organismal, population, community, and ecosystem responses, each with its particular experimental methodologies and suite of specialized instrumentation, equipment, facilities, and experimental protocols. These investigations generate data; whereas 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. 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 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 objective is essential for determining dose-response functions used in the development of impact and risk assessments of the effects of O_3 and usually involves treating plants to a range of artificial O_3 exposures. The second objective is essential for determining the geographic distribution of the risk and usually involves plants subjected 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. Methods used to characterize the O_3 content of ambient air and to define exposure and exposure-response relations are discussed in Sections 9.5 and 9.6, respectively.

The methodologies for exposure-response studies have used many different types of exposure facilities and protocols and have employed a range of statistical approaches to the analysis and interpretation of 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

1   advances has been the clearer understanding that has emerged from earlier work identifying the

2   *type* of experimentation needed to achieve realistic assessments of the magnitude and extent of

3   the impact of O_3 on vegetation of all types. As a result, significantly increased attention is now

4   being paid to field observations of responses to ambient O_3 pollution, particularly to the

5   responses of forest trees and native vegetation.

6   Other than in various exploratory studies that have used chamber-based steady-state

7   exposure concentrations (so-called "square-wave" exposures), the trend in experimental

8   exposure protocols has been to attempt to expose plants under conditions as natural as possible

9   to temporal profiles that simulate the real-world, either by conducting experiments in the field or

10 in elaborately controlled environment facilities that provide simulated field conditions.

11   Previous Air Quality Criteria Documents for Ozone and Other Photochemical Oxidants

12 (U.S. Environmental Protection Agency, 1986; 1996) described the time-course for these

13 methodological developments. Although this section provides a brief overview of the

14 methodologies used in the past, and their limitations, it focuses mainly on those techniques that

15 have come into prominence over the last decade. This has been aided considerably by several

16 compilations of experimental methodologies and facilities, such as the earlier comprehensive

17 review for the U.S. Environmental Protection Agency/National Acid Precipitation Assessment

18 Program by Hogsett et al. (1987a; 1987b) and the more recent reviews by Manning and Krupa

19 (1992), Musselman and Hale (1997) and Karnosky et al. (2001).

20

21 ## 9.2.2 Methods Involving Experimental Exposures to O_3

22 ### 9.2.2.1 "Indoor", Controlled Environment, and Greenhouse Chambers

23   The earliest experimental investigations of the effects of O_3 on plants utilized simple glass

24 or plastic-covered chambers, often located within greenhouses, into which a flow of O_3-enriched

25 air or oxygen could be passed to provide the exposure. The types, shapes, styles, materials of

26 construction, and locations of these chambers were as numerous as the different investigators

27 and, in spite of providing little resemblance to real-world conditions, they yielded much of the

28 basic information on the visible and physiological effects on plants. The construction and

29 performance of more elaborate and better instrumented chambers dating back to the 1960s has

30 been well-summarized in Hogsett et al. (1987a), including those installed in greenhouses (with

31 or without some control of temperature and light intensity).

1   One greenhouse chamber approach that continues to yield useful information on the

2   relationships of O_3 uptake to both physiological and growth effects employs continuous stirred

3   tank reactors (CSTRs) first described by Heck et al. (1978). Although originally developed to

4   permit mass-balance studies of O_3 flux to plants, their use has more recently widened to include

5   short-term physiological and growth studies of $O_3 \times CO_2$ interactions (e.g., Costa et al., 2001;

6   Heagle et al., 1994b; Loats and Rebbeck, 1999; Rao et al.. 1995; Reinert and Ho. 1995; and

7   Reinert et al.. 1997), and of surveys of native plant responses to O_3 (Orendovici et al., 2003).

8   In many cases, supplementary lighting and temperature control of the surrounding structure have

9   been used to control or modify the environmental conditions, e.g., Heagle et al. (1994a).

10   Many investigations have utilized commercially available controlled environment

11   chambers and walk-in rooms adapted to permit the introduction of a flow of O_3 into the

12   controlled air-volume. Such chambers continue to find use in genetic screening and in

13   physiological and biochemical studies aimed primarily at improving our understanding of mode

14   of action. For example, some of the ongoing studies of the O_3 responses of *Plantago major*

15   populations have been conducted in controlled environment chambers (Reiling and Davison,

16   1994; Whitfield et al., 1996b).

17   The environmental conditions provided by indoor chambers of any type will always

18   preclude the use of the information obtained with such chambers in predicting O_3 effects in the

19   natural environment, because the environmental conditions will always be measurably different

20   from field conditions. However, highly sophisticated controlled environment chambers such as

21   those described by Langebartels et al. (1997), which are subdivided into aerial and root

22   compartments, with dynamic control of light intensity and photoperiod, air and soil temperature,

23   humidity, soil moisture, wind speed, and exposure to O_3, may come close to simulating specific

24   natural conditions. Such chambers have provided meaningful insights into a wide array of early

25   biochemical responses of plants to O_3. They can minimize confounding factors that make indoor

26   chamber studies only rarely able to be extrapolated to field conditions, i.e., that shoots and roots

27   develop under different temperature regimes.

28   The applicability of the results of many chamber studies is also limited by their use of

29   container-grown plants. Several recent studies have raised serious questions as to the relevance

30   of pot-based studies to the true field situation (even when the studies are conducted in the field.)

31   Most of the questions have concerned studies of the effects of CO_2 enrichment, as discussed in

1   Section 9.4.7.1, but are also relevant to O_3 enrichment studies, as shown by Whitfield et al.

2   (1996a). Whitfield et al., reported significant interactive effects between O_3 and soil volume on

3   the growth of *Plantago major*. They noted that although container size may limit root and,

4   hence, plant growth, the reverse may also be true for single plants in large containers which do

5   not experience typical field competition for resources.

6

7   **9.2.2.2   Field Chambers**

8   Although closed field chambers have largely fallen out of favor in recent years, closed

9   "Solardome" field chambers (Lucas et al., 1987; Rafarel and Ashenden, 1991) have been used

10   recently in studies of $O_3 \times$ acid mist interactions (Ashenden et al., 1995; 1996).

11   Concern over the need to establish realistic plant-litter-soil relationships as a prerequisite to

12   studies of the effects of O_3 and CO_2 enrichment on ponderosa pine (*Pinus ponderosa*) seedlings

13   led Tingey et al. (1996) to develop closed, partially environmentally controlled, sun-lit chambers

14   ("terracosms") incorporating 1 m-deep lysimeters containing forest soil that retained the

15   appropriate horizon structure.

16   In general, field chamber studies are dominated by the use of various versions of the open-

17   top chamber (OTC) design, first described by Heagle et al. (1973) and Mandl et al. in 1973.

18   Most chambers are ~3 m in diameter with 2.5 m high walls. Hogsett et al. (1987a) described in

19   detail many of the various modifications to the original OTC designs that appeared subsequently,

20   e.g., the use of larger chambers to permit exposing small trees (Kats et al., 1985); and grapevines

21   (Mandl et al., 1989), the addition of a conical baffle at the top to improve ventilation (Kats et al.,

22   1976), a frustrum at the top to reduce ambient air incursions, and a plastic rain-cap to exclude

23   precipitation (Hogsett et al., 1985). All of these modifications included the discharge of air via

24   ports in annular ducting or interiorly perforated double-layered walls at the base of the chambers

25   to provide turbulent mixing and upward mass flow of air.

26   Wiltshire et al. (1992) described a large open-top chamber suitable for small trees with

27   roll-up sides that permitted the trees to be readily subjected from time to time to episodic,

28   normal, "chamberless" environmental conditions. In the 6 m-high OTCs described by Seufert

29   and Arndt (1985) used with Norway spruce (*Picea abies*) trees, a second zone of annular

30   enrichment was also provided between 4 and 5 m. The use of OTCs was adopted for the large

31   European Stress Physiology and Climate Experiment on the effects of CO_2 and O_3 on spring

1   wheat (ESPACE-wheat), conducted in 1994-1996 at field sites in eight countries (Jäger et al.,

2   1999). However, typical European chambers have the introduction of O_3-enriched air at or

3   above canopy height. The relatively low costs of fabrication, operation and maintenance has

4   favored OTC use in field studies (Fangmeier et al., 1992; Musselman and Hale, 1997). The air

5   supplied to the chambers can be readily filtered through activated charcoal to reduce the O_3

6   concentration or it can be enriched with O_3 to provide a range of exposures.

7   All field chambers create internal environments that differ from ambient air, giving rise to

8   so-called "chamber effects" with the modification of microclimatic variables, including reduced

9   and uneven light intensity, uneven rainfall, constant wind speed, reduced dew formation, and

10   increased air temperatures (Fuhrer, 1994; Manning and Krupa, 1992). Several shortcomings of

11   the OTC design and operation relate to the means of introduction and mixing of enriched air to

12   produce a definable exposure. First, the plants are subjected to constant turbulence, which,

13   through increased uptake resulting from the consequently low boundary layer resistance to

14   diffusion, may lead to overestimation of the magnitude of real-world cause-effect relationships

15   (Krupa et al., 1995; Legge et al., 1995). Second, the introduction of the O_3-enriched air in the

16   lower part of chambers described by Heagle et al. (1973) and Mandl et al. (1973) results in a O_3

17   concentration gradient that decreases with increasing height, the converse of the situation

18   observed in ambient air, in which the O_3 concentration decreases markedly from above a plant

19   canopy to ground level (Grünhage and Jäger, 1994; Pleijel et al., 1995; Pleijel et al., 1996).

20   Concern that studies conducted in such OTCs may somewhat overestimate the effects of O_3 led

21   to the European design which provides a decreasing downward gradient. It seems unlikely that

22   the "chamber effects" produced by the two designs will be the same. These issues are discussed

23   more fully in Section 9.2.2.4.

24   It should also be noted that, although originally developed for exposing row drops in the

25   field, many recent studies employing OTCs have used potted plants in order to include or control

26   edaphic or nutritional factors or water relations within the experimental design. Therefore, the

27   same caveats as those discussed above (Section 9.2.2.1) with regard to extrapolating results of

28   pot studies to true field conditions apply to OTC studies.

29   The difficulties faced in the experimental exposure of forest trees to air pollutants in

30   chambers (e.g., Seufert and Arndt [1985]) led to the development of branch chambers such as

31   those described by Ennis et al. (1990), Houpis et al. (1991) and Teskey et al. (1991). These

1 chambers are essentially large cuvettes and, as noted by Musselman and Hale (1997), share

2 many of the characteristics of CSTRs, i.e., transparent walls, internal fans, and inlet and outlet

3 monitors to permit the determination of O_3 uptake, CO_2 exchange, and transpiration. Although

4 they make it possible to expose whole branches to different O_3 regimes, the relevance of the data

5 they yield in regard to the whole tree may be questioned. As noted by Saxe et al. (1998), the

6 inevitable change in environmental conditions resulting from the isolation of the branch may

7 cause different responses from those that would be obtained if the whole tree was subjected to

8 the same environmental conditions.

9

10 **9.2.2.3 Plume Systems**

11 Plume systems are chamberless exposure facilities in which the atmosphere surrounding

12 plants in the field is modified by the injection of pollutant gas into the air above or around them

13 from multiple orifices that are spaced to permit diffusion and turbulence so as to establish

14 relatively homogeneous conditions as the individual plumes disperse and mix with the ambient

15 air. As pointed out by Manning and Krupa (1992), they can only be used to *increase* the O_3

16 levels in the ambient air. The volume of air to be modified is unconfined, and three approaches

17 have been used to achieve desired pollutant concentrations in the air passing over the plants

18 producing various systems that:

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

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

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

22 Gradient systems created by dispensing a pollutant gas into the air at canopy level from

23 perforated horizontal pipes arranged at right angles to the prevailing wind were described for

24 SO_2 studies in the early 1980s. A modified gradient system for O_3 was used by Bytnerowicz

25 et al. (1988) to study effects on desert species, but there appear to have been no recent

26 applications of the method. A gradient O_3-*exclusion* system is discussed in Section 9.2.3.1.

27 Systems designed to achieve spatially uniform pollutant levels by ensuring that the release

28 of a pollutant is always on the upwind side of the study site were also originally described for

1   SO$_2$ studies, e.g. Greenwood et al. (1982). However, the adaptation of these concepts as

2   introduced by McLeod et al. (1985) in constructing a large circular field site for exposing crops

3   to SO$_2$ led to the subsequent development of both the large-scale O$_3$ and SO$_2$ fumigation system

4   for forest trees in the United Kingdom in 1985 (the Liphook Forest Fumigation Project; McLeod

5   et al., 1992), the smaller system for O$_3$ fumigation constructed at Kuopio, Finland in 1990

6   (Wulff et al., 1992), and the free-air carbon-dioxide enrichment (FACE) systems of gas dispersal

7   over crops (Hendrey and Kimball, 1994) and forest trees (Hendrey et al., 1999). Although

8   originally designed to provide chamberless field facilities for studying the CO$_2$ effects of climate

9   change, large forest tree FACE systems have recently been adapted to include the dispensing of

10 O$_3$ (Karnosky et al., 1999). Volk et al. (2003) have recently described a system for exposing

11 grasslands that uses 7-m diameter plots. FACE systems discharge the pollutant gas (and/or CO$_2$)

12 through spaced orifices along an annular ring (or torus) or at different heights on a ring of

13 vertical pipes. Computer-controlled feedback from the monitoring of gas concentration

14 regulates the feed rate of enriched air to the dispersion pipes. Feedback of wind speed and

15 direction information ensures that the discharges only occur upwind of the treatment plots, and

16 that discharge is restricted or closed down during periods of low wind speed or calm conditions.

17 The diameter of the arrays and their heights in some FACE systems (25-30 m) requires large

18 throughputs of enriched air per plot, particularly in forest tree systems. The cost of the

19 throughputs tends to limit the number of enrichment treatments, although Hendrey et al. (1999)

20 have argued that the cost on an enriched volume basis is comparable to that of chamber systems.

21   An alternative to the FACE system to free-air fumigation uses a horizontal grid system

22 through which pollutant-air enriched is discharged over the canopies of plants in field plots. The

23 original design, termed the Zonal Air Pollution System (ZAPS), was developed for studying the

24 effects of SO$_2$ on native grasslands (Lee et al., 1975), and it was later modified by Runeckles

25 et al. (1990) by randomly dividing each of three treatment plots into four sub-plots, each with

26 different numbers of discharge orifices to provide various levels of O$_3$ enrichment. With the

27 ZAPS system, changes in wind direction and speed result in varying degrees of carry-over from

28 sub-plot to sub-plot, effectively resulting in twelve stochastically different seasonal exposures.

29 The system was used for studies of growth effects on field crops and 2- to 4-year old Douglas fir

30 (*Pseudotsuga menziesii*) saplings (Runeckles and Wright, 1996).

1   A larger ZAPS design was used by Wilbourn et al. (1995) on a grass (*Lolium perenne*)-

2   clover (*Trifolium repens*) mixture and by Ollerenshaw et al. (1999) on oilseed rape (*Brassica*

3   *napus*), whereby four replicate field plots were exposed to intermittent constant additions of O_3

4   to ambient air. A ZAPS design with eight spatially separated treatment plots was also developed

5   to obtain crop response data used in assessing crop losses in the Fraser Valley, British Columbia,

6   Canada (Runeckles and Bowen, 2000).

7   The FACE-type facility developed for the Kranzberg Ozone Fumigation Experiment in

8   Germany that began in 2000 (KROFEX; Werner and Fabian, 2002; Nunn et al., 2002) to study

9   the effects of O_3 on mature stands of beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees is

10   more truly a zonal system that functions independently of wind direction. The enrichment of a

11   large volume of the ambient air immediately above the canopy takes place via orifices in vertical

12   tubes suspended from a horizontal grid supported above the canopy.

13   Recognizing the difficulties of modifying the aerial environments of large trees, Tjoelker

14   et al. (1994) devised a free-air system for exposing branches of sugar maple (*Acer saccharum*)

15   trees to O_3. Near the ends of up to 10 branches, enriched air was discharged through small holes

16   in 38-cm-diameter loops of 0.635-cm-OD teflon tubes positioned 20-30 cm below the terminal

17   foliage cluster.

18   Although plume systems make virtually none of the modifications to the physical

19   environment that are inevitable with chambers, their successful use depends on selecting the

20   appropriate numbers, sizes, and orientations of the discharge orifices to avoid "hot-spots"

21   resulting from the direct impingement of jets of pollutant-enriched air on plant foliage (Werner

22   and Fabian, 2002). However, because mixing is unassisted and completely dependent on wind

23   turbulence and diffusion, local gradients are inevitable even in large-scale FACE systems. Both

24   FACE and ZAPS systems have provisions for shutting down under low wind speed or calm

25   conditions and for an experimental area that is usually defined within a generous border, in order

26   to strive for homogeneity of the exposure concentrations within the treatment area. They are

27   also both dependent upon continuous computer-controlled feedback of the O_3 concentrations in

28   the mixed treated air and the meteorological conditions.

29

30

9.2.2.4 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 consistent turbulence over and within the plant canopy during chamber operation, in modifying plant responses.

With the widespread use of the OTC, numerous studies have demonstrated increased air temperatures and decreased light intensities as regularly noted features of their use. However, the question to be answered is whether or not these differences affect plant response to O_3. As noted in the 1996 criteria document (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." Several more recent studies have, however, indicated that the temperature effect alone may be sufficient to cause significant (though modest) shifts in quantitative responses to O_3, both positive and negative, as discussed below in Section 9.4.3.2. 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 in large OTCs to determine if "insidious differences in microclimatic conditions could alter plant growth responses and susceptibility to pollutant stress." Non-filtered chambers were found to have somewhat lower average O_3 concentrations than the ambient air, with 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 avoid automatically assuming that OTCs yield response data that are all immediately relevant to the real world, particularly since, as in this study, no O_3 enrichment was involved as a complicating factor.

1   Plume systems avoid "chamber effects," but because they rely solely upon diffusion and

2   natural turbulence to modify the ambient O_3 concentration, they may fail to achieve homogeneity

3   of the air to which the plants are exposed and may give rise to "hot spots" in which the enriched

4   air jets are inadequately diluted and impinge directly on foliage. A further deterrent to their

5   widespread use is the large-scale generation of O_3 needed, which has in most cases limited the

6   numbers of treatments that can be included in an experimental design. In spite of the various

7   advantages and disadvantages of the two field systems, it appears that no direct comparative

8   studies have been conducted.

9

10   **9.2.2.5 Ozone Generation Systems**

11   Two approaches have been used to generate the O_3 needed for enrichment purposes from

12   air or oxygen: (1) high voltage static discharge and (2) high intensity UV-irradiation. Using

13   gaseous oxygen as feedstock, both generate O_3-enriched oxygen, free from other impurities.

14   However, the use of high voltage discharge equipment with air as feedstock requires that the

15   output be scrubbed with water to remove appreciable amounts of the higher oxides of nitrogen

16   (especially N_2O_5, nitric acid vapor) that form concurrently with O_3 (Brown and Roberts, 1988;

17   Taylor et al., 1993).

18

19   **9.2.2.6 Experimental Exposure Protocols**

20   A few recent chamber studies of physiological or biochemical effects have continued to

21   use "square-wave" exposure profiles typified by a rapid rise to and falling off from a steady

22   target concentration. However, most recent experimentations into O_3 effects on plant growth and

23   development have employed either simulations of the diurnal ambient O_3 profile or

24   enhancement/reduction of the ambient O_3 concentrations.

25   Although Hogsett et al. (1985), Lefohn et al. (1986), and others have described the use in

26   controlled chambers of daily exposure profiles based on observed ambient O_3 profiles and such

27   profiles were used in the elaborately controlled chamber studies of Langbartels et al. (1997),

28   several recent chamber studies have used simpler computer-controlled half- or full-cosine wave

29   profiles to simulate the typical daily rise and fall in ambient O_3 levels (McKee et al., 1997a;

30   1997b; Mazarura, 1997).

1   The early studies with OTCs involved adding constant levels of O_3 to ambient air O_3

2   concentrations, but all recent studies have used enrichment delivery systems that maintain

3   proportionality to and track ambient O_3 concentrations to produce levels that more closely

4   resemble field observations. Both FACE and ZAPS studies have used proportional enrichment

5   to provide a range of treatments, although Wilbourn et al. (1995) and Ollerenshaw et al. (1999)

6   adjusted their systems manually to obtain a relatively constant target concentration during

7   exposure episodes.

8

9   ## 9.2.3 Methods Involving Exposures to O_3 in Ambient Air

10   The experimental methods discussed above are largely aimed at developing quantitative

11   growth-response functions to permit the estimation of the effects of different ambient O_3

12   scenarios. Because such methodologies usually involve exposures to higher than ambient O_3

13   levels, the applicability of the functions obtained may, to some extent, be relevant only to

14   locations that are naturally subjected to high ambient O_3 levels. Furthermore, as pointed out in

15   Section 9.4, the response functions that they generate rarely incorporate other environmental,

16   genetic, and physiological factors, many of which can severely modify the magnitude of the

17   response to O_3. The consequences of ignoring such modifications have been well stated by De

18   Santis (1999). The European standard for protecting crops (based on the AOT40 index) was

19   derived from OTC studies of O_3-induced grain loss of wheat observed in experiments conducted

20   mostly in non-Mediterranean locations. However, the impact of ambient O_3 on wheat yields in

21   the Po Valley of northern Italy is much less than the devastatingly high loss (> 60%) suggested

22   by the seasonal exceedances of the standard. On a similar note, Manning (2003) has recently

23   urged the absolute necessity of seeking "ground truth" as verification of the nature and

24   magnitude of impacts on vegetation suggested by response functions using ambient O_3

25   monitoring data.

26   Such concerns clearly show that attention needs to be focused on incorporating

27   consideration of environmental and other factors into the response functions upon which

28   standards are based. This will require the development of improved simulation response models.

29   These concerns have also led to increasing attention being paid to seeking and developing

30   alternative approaches to the assessment of impact and the geographic extent of such impact,

31   approaches that are based on *in situ* exposures to ambient or sub-ambient O_3 levels. Although

1   one approach, the use of air exclusion systems, requires experimental facilities, the other

2   approaches are generally based on simple field observations or measurements and, hence, can be

3   undertaken on a wide geographic scale.

4

5   **9.2.3.1 Air-exclusion Systems**

6   The term, air-exclusion system, usually refers to chamberless field systems specifically

7   designed to protect plants from exposure to polluted air by blowing filtered air through their

8   canopies. Hogsett et al. (1987a; 1987b) describe several dedicated systems developed in the

9   1960s and 1970s, but there appear to have been no recent O_3-exclusion studies using systems

10 specifically designed for the purpose since those described by Olszyk et al. (1986a; 1986b).

11 Their system, a modification of the earlier system of Jones et al. (1977), consisted of perforated

12 31.8-cm OD inflatable polyethylene tubes laid between crop rows and supplied with charcoal-

13 filtered air. By increasing the size of the orifices progressively in sections along the 9-m length

14 of the tubes, an exclusion gradient was created with a progressive decrease in O_3 levels in the air

15 surrounding the crop from one end of the system to the other. The system was used for studies

16 on alfalfa (*Medicago sativa L.*) comparing plant response in OTCs, closed field chambers, the

17 air-exclusion system, and ambient air plots (as discussed above in Section 9.2.2.4).

18   An air-exclusion component has also been part of the overall design of the many OTC

19 experiments. Charcoal-filtered air or mixtures of charcoal-filtered and ambient air to chambers

20 as part of the overall design.

21

22 **9.2.3.2 Natural Gradients**

23   Naturally occurring locational differences in ambient O_3 concentrations hold potential for

24 the examination of plant response along a gradient of such concentrations. However, few such

25 gradients can be found which meet the rigorous statistical requirements for comparable site

26 characteristics such as soil type, temperature, rainfall, radiation, and aspect (Manning and Krupa,

27 1992), although with small plants, soil variability can be avoided by the use of potted plants.

28   Studies in the 1970s used the natural gradients occurring in Southern California to assess

29 yield losses of alfalfa (saliva) and tomato (*Lycopersicon esculentum L.*) (Oshima et al., 1976;

30 1977). A transect study of the impact of O_3 on the growth of clover (*Trifolium repens L.*) and

31 barley (*Hordeum vulgare L.*) in the United Kingdom was confounded by differences in the

1 concurrent gradients of SO_2 and NO_2 pollution (Ashmore et al., 1988). Studies of forest tree

2 species in national parks in the eastern U.S. (Winner et al., 1989) revealed increasing gradients

3 of O_3 and visible foliar injury with increased elevation.

4

5 **9.2.3.3   Use of Chemical Protectants**

6   The use of protective chemicals is a relatively inexpensive, promising alternative to

7 experimental field exposures in chambers or free-air systems for determining plant response to

8 O_3. Several chemical compounds (antioxidants, antisenescence agents, fungicides, pesticides,

9 etc.) have been known for many years to provide plants some protection from photochemical

10 oxidants, such as O_3 (Manning and Krupa, 1992). Most of these chemicals were originally used

11 as a one-time application to reduce visible injury caused by acute O_3 exposures. The most

12 widely used and popular of these has been ethylenediurea (EDU). Carnahan et al. (1978)

13 reported that EDU protected pinto bean (*Phaseolus vulgaris*) from acute O_3 injury. After this

14 initial investigation, EDU was shown to suppress visible O_3 injury on several species of plants

15 under both controlled and field conditions (Brennan et al., 1987; Clarke et al., 1983). However,

16 due to lack of a commercial market for this product, its commercial manufacture was largely

17 discontinued. Other chemicals, including benomyl (Manning et al., 1974), carboxin (Rich et al.,

18 1974), ascorbic acid (Dass and Weaver, 1968) and others (Manning and Krupa, 1992), exhibited

19 some beneficial effects in reducing visible O_3 injury.

20   Despite their widespread use for O_3-effects studies, OTCs are not effective in determining

21 plant responses in the field under truly natural conditions, as discussed above, and are not

22 efficient or easily used in unmanaged ecosystems such as wilderness areas (Manning and Krupa,

23 1992). For these reasons, a renewed interest in using protective chemicals as research tools

24 occurred in the early 1990s. These chemicals (mostly EDU) have recently been used in studies

25 of different plant species, both in the United States (Bergweiler and Manning, 1999; Kuehler and

26 Flagler, 1999) and in Europe (Bortier et al., 2001b; Pleijel et al., 1999; Wu and Tiedemann,

27 2002), to determine if ambient O_3 concentrations affect plant growth and productivity or are just

28 exacerbating foliar injury. For example, Bortier et al. (2001a) injected seedlings of an O_3-

29 sensitive poplar (*Populus nigra*) clone with EDU and measured growth over a 1-year period at a

30 field site near Brussels, Belgium. Over the growing season, stem diameter increment was

31 significantly higher (16%), biomass was increased (9%), and foliar O_3 symptoms were slightly

1   less for the EDU-treated seedlings. Ozone levels were reported to be low (AOT40 = 6170 ppb.h,

2   May-September) during the exposure period. In another study, Manning et al. (2003) applied

3   EDU (foliar spray) and sodium erythorbate (NaE) at various concentrations, biweekly for three

4   growing seasons to loblolly pine (*Pinus taeda*) at a field site in east Texas. After 3 years, the

5   trees were harvested and biomass measured. Neither EDU nor NaE prevented foliar O_3 injury,

6   but EDU applications at 450 ppm resulted in increases in stem diameter and height and total

7   above-ground biomass. These measures of growth also tended to slightly increase with

8   applications of NaE, but the effects were nonsignificant.

9   Several recent studies used EDU in assessing the response of several plant species to O_3 to

10   help validate the proposed critical level (AOT40 = 3000 ppb.h) for crop protection in Europe

11   (Ball et al., 1998; Ribas and Penuelas, 2000; Tonneijck and Van Dijk, 2002b; 2002a). EDU

12   appeared to provide protection from visible foliar injury, but the results regarding yield and

13   biomass reductions were mixed. In a 3-year study over 12 sites throughout Europe, Ball et al.

14   (1998) used the ratio of EDU-treated versus non-treated white clover (*Trifolium repens*)

15   biomass but did not find a significant relationship between biomass reductions and AOT40.

16   When other parameters, such as temperature and VPD were included in the model however,

17   they found a significant relationship ($r^2 = 0.79$) between biomass reductions and AOT40, and

18   the greatest biomass reductions occurred in areas with the highest levels of industrialization

19   (in Germany).

20   Tonneijck and Van Dijk (2002b) assessed the relationship of visible injury of subterranean

21   clover (*Trifolium subterraneum*) to ambient O_3 at four sites over three growing seasons in the

22   Netherlands, using EDU-treated and non-treated plants. Visible injury varied by site and year,

23   but was reduced to near zero by EDU treatment. However, no relationship indicative of a

24   protective effect of EDU with this plant species was observed for biomass. Tonneijck and

25   Van Dijk (2002a) also reported similar results with bean (*Phaseolus vulgaris*). Both EDU-

26   treated and non-treated plants were exposed to ambient O_3 at three locations in Spain over one

27   growing season (Ribas and Penuelas, 2000). Reductions in yield and biomass were correlated

28   with O_3 concentration and EDU provided some protective effect, although results varied by

29   location and with meteorological conditions.

30   The mechanisms by which protective chemicals, especially EDU, protect plants are poorly

31   understood. However, Wu and von Tiedemann (2002) reported that applications of two recently

1 developed fungicides (azoxystrobin and epoxiconazole) provided protection of spring barley to

2 relatively high O_3 exposures (150-250 ppb, 5 days, 7h/d) and resulted in increases in leaf soluble

3 protein content, as well as the activity of several antioxidative enzymes (e.g., superoxide

4 dismutase, catalase, ascorbate-peroxidase, and glutathione reductase). In addition, the increase

5 in these enzymes reduced superoxide levels in the leaves.

6 Before using these chemicals in a field setting, preliminary investigations should be done

7 to evaluate the methods and timing of application, as well as proper application rates, so as to

8 avoid any potential toxic effects (Manning, 2000; Manning and Krupa, 1992). Also, because

9 certain plant species may be nonresponsive, careful pre-screening of various species to be tested

10 is critical.

11 In summary, the use of protectants such as EDU (see Table 9-1) provides a ready means of

12 demonstrating the local occurrence of adverse effects of O_3, both in terms of visible injury and

13 growth reduction. However, even if accompanied by some type of O_3 and meteorological

14 monitoring, their use has yet to give rise to a methodology for quantitatively assessing exposure

15 response.

16

17 **9.2.3.4 Biomonitoring**

18 ***Bioindicators***

19 The use of biological indicators to detect the presence of O_3 injury to plants is a

20 longstanding and effective methodology (Chappelka and Samuelson, 1998; Manning and Krupa,

21 1992). A bioindicator can be defined as a vascular or non-vascular plant exhibiting a typical and

22 verifiable response when exposed to a plant stress such as an air pollutant (Manning et al., 2003).

23 To be considered a good indicator species, plants must:

24 (1) exhibit a distinct, verified response;

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

26 (3) exhibit genetic stability.

27 Such sensitive plants can be used to detect the presence of a specific air pollutant such as

28 O_3 in the ambient air at a specific location or region and, as a result of the magnitude of their

29 response, provide unique information regarding specific ambient air quality. Bioindicators can

30 be either introduced *sentinels,* such as the widely used tobacco (*Nicotiana tabacum*) variety

Table 9-1. Advantages and Disadvantages of Protective Chemicals Used in Assessment of O₃ Effects on Plants

Advantages
No chambers required. Plants exposed to ambient conditions of O_3, light, temperature, etc.
Can conduct studies "in situ." Equipment needs are minimal. No "chamber effects"
A high degree of replication possible both within and among locations

Disadvantages
Exposure-response studies require inclusion of other methodologies (OTCs, etc.)
Need measurements of ambient O_3 and other meteorological variables (temp, rainfall, etc)
Have to conduct preliminary toxicology studies to determine proper rate, timing etc.
Possible plant toxicity can result from repeated applications
Species response can vary; need to screen for proper species to use

Modified from Manning and Krupa (1992).

1 Bel W3, or *detectors,* which are sensitive native plant species. The approach is especially useful

2 in areas where O_3 monitors are not operated (Manning et al., 2003). For example, in remote

3 wilderness areas where instrument monitoring is generally not available, the use of bioindicator

4 surveys in conjunction with the use of passive samplers (Krupa et al., 2001) is a particularly

5 useful methodology (Manning et al., 2003). However, the method requires expertise or training

6 in recognizing those signs and symptoms uniquely attributable to exposure to O_3 as well as their

7 quantitative assessment.

8   Since the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) many new

9 sensitive species have been identified from controlled exposure studies and verified in the field

10 (Flagler, 1998; Innes et al., 2001). In addition, several new uses of this methodology have been

11 demonstrated, including a national O_3 bioindicator network, studies in wilderness areas, and

12 mature tree studies. Although it has been difficult to find robust relationships between the foliar

13 injury symptoms caused by O_3 and effects on plant productivity or ecosystem function, visible

14 injury correlations with growth responses have been reported (Table 9-2; Chappelka and

15 Samuelson, 1998; Manning et al., 2003; Smith et al., 2003). One workshop on the utility of

16 bioindicators of air pollutants led to a useful series of peer-reviewed publications in

17 *Environmental Pollution* (Skelly, 2003).

Table 9-2. 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
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
Use more than one indicator species (detector) per area if possible
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 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 U.S. (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 (1994-1999) from the network to identify several species that were sensitive to O$_3$ over a regional scale

1   included sweetgum *(Liquidambar styraciflua)*, loblolly pine (*Pinus Taeda*), and black cherry

2   (*Prunus serotina*).

3

4   ***Wilderness areas***

5   The use of bioindicator species as detectors has proven to be an effective technique for

6   deriving a relative estimate of O_3 injury in wilderness areas in both the US and Europe

7   (Chappelka et al., 1997; 2003; Manning et al., 2002). However, to be truly effective, these

8   regional and national bioindicator studies need the inclusion of air quality data and related

9   growth studies to determine effects on productivity and ecosystem function (Bytnerowicz et al.,

10   2002; Manning et al., 2003; Smith et al., 2003).

11   Chappelka et al. (1997; 2003) conducted surveys of foliar injury on several native plant

12   species throughout the Great Smoky Mountains National Park (GRSM), including black cherry

13   (*Prunus serotina*), tall milkweed (*Ascelpias exaltata*), cutleaf coneflower (*Rudbeckia laciniata*)

14   and crownbeard (*Verbesina occidentalis*). Visible foliar symptoms were prevalent throughout

15   the Park, indicating that injury-producing O_3 levels were widespread in GRSM.

16   Manning et al. (2002) recently summarized a multi-year (1993-2000) bioindicator project

17   in the Carpathian Mountain range in eastern Europe. They evaluated numerous trees, shrubs,

18   forbs, and vines for possible symptoms of O_3 injury. Observations were made at plots located in

19   the vicinity of either active or passive O_3 monitors (Bytnerowicz et al., 2002). Approximately 30

20   species of native plants detectors were identified as possible bioindicators, the majority of which

21   (21) were shrubs (Manning et al., 2002). Based on these observations, it was concluded that O_3

22   concentrations were sufficiently high to impact ecosystems in the region. Similar investigations

23   regarding the sensitivity of native species have been conducted in Switzerland (Novak et al.,

24   2003) and Spain (Orendovici et al., 2003).

25

26   ***Mature tree detectors***

27   Many studies have reported visible injury of mature coniferous trees caused by O_3,

28   primarily in the western United States (Arbaugh et al., 1998) and, to a lesser extent, to mature

29   deciduous trees in eastern North America. In an effort to determine the extent and magnitude of

30   visible injury in mature tree canopies, Hildebrand et al. (1996) and Chappelka et al. (1999b)

31   conducted independent studies in the GRSM and the Shenandoah National Park (SHEN). The

1   species examined were sassafras (*Sassafras albidum*), black cherry, and yellow-poplar

2   (*Liriodendron tulipifera* L.) in GRSM and white ash *(Fraxinus americana L.)*, black cherry, and

3   yellow-poplar in SHEN. Protocols were similar at both parks, and trees were located near O_3

4   monitors at three different areas in each park. Results from both studies indicated that symptoms

5   of O_3 injury were present in the trees and correlated with the amount of injury both spatially and

6   temporally. Ozone injury tended to be most severe at the highest elevation, except with yellow-

7   poplar.

8   Hildebrand et al. (1996) observed significant O_3 exposure-plant response relationships with

9   black cherry. The best relationships were found between foliar injury and the SUM06 and W126

10   exposure indices, indicating that higher O_3 concentrations were important in eliciting a response

11   in black cherry. No O_3 exposure-plant response relationships were found with any species tested

12   in GRSM (Chappelka et al., 1999b); but, when the data were combined for both parks, a

13   significant correlation r = 0.72) with black cherry was found for both SUM06 and W126, and

14   injury was the greatest (r = 0.87) at the higher elevations (Chappelka et al., 1999a).

15   Based on a study in which visible symptoms of O_3 injury were characterized for large,

16   mature yellow-poplar and black cherry trees in GRSM (Chappelka et al., 1999a), Somers et al.

17   (1998) compared radial growth differences among trees classified as sensitive or non-sensitive

18   based on the severity of visible foliar injury observed over a 3-year period (1991-1993).

19   Significantly more radial growth was observed over both a 5- and a 10-year period for the non-

20   sensitive compared to the sensitive trees. No significant relationship was found for black cherry

21   tree growth.

22   Vollenweider et al. (2003), using data collected from continuous forest inventory (CFI)

23   plots across Massachusetts, compared growth rates among either symptomatic or asymptomatic

24   mature black cherry trees. Of the 120 trees sampled in 1996, 47% exhibited visible foliar injury.

25   Using CFI data, growth rates were compared over a 31-year period. The growth rates for

26   symptomatic trees were reduced by 28% compared with the asymptomatic trees.

27   Because these studies (Somers et al., 1998; Vollenweider et al., 2003) were not controlled

28   studies and used a small sample of trees, they cannot validly be used to characterize cause and

29   effects related to the visible symptoms and radial growth they describe. However, the results

30   indicate the *possibility* that O_3 is correlated with growth losses in some sensitive genotypes,

1   illustrating the potential usefulness of this visible O_3 injury methodology in assessing effects on

2   the growth rates of mature deciduous trees.

3

4   ***Cultivar Comparisons***

5   The idea of using cultivars or isogenic lines of crop species that differed in O_3 sensitivity as

6   sentinels to determine the ambient effects of O_3 in the field was presented in the 1996 O_3 AQCD

7   (U.S. Environmental Protection Agency, 1996). The rationale was that comparing the ratio of

8   injury scores or some measure of growth between two different cultivars varying in O_3

9   sensitivity should be indicative of the relative amount of ambient stress to plants at a given

10   location. A sensitive:resistant ratio close to unity would indicate relatively low O_3

11   concentrations and a low ratio higher O_3 levels. Results from locations differing in O_3

12   concentrations could be evaluated to develop exposure-response models. The original protocol

13   was derived using two isogenic lines of white clover (*Trifolium repens*) differing in O_3

14   sensitivity (Heagle et al., 1994b; 1995).

15   This white clover model system has been used in several multi-location studies in the U.S.

16   (Heagle and Stefanski, 2000) and Europe (Ball et al., 2000; Bermejo et al., 2002; Mills et al.,

17   2000). Heagle and Stefanski (2000) compared results from eight sites over a 2-year period with

18   various exposure indices (SUM00, SUM06, W126 and others) to determine a best-fit regression.

19   They found that most of the indices preformed similarly. The highest r^2 values (0.87-0.93) were

20   obtained using only the later harvests and a 6 h d^{-1} index (1000-1600 h). Similar multiple-

21   comparison studies conducted in Europe using the AOT40 index (Ball et al., 2000; Mills et al.,

22   2000) yielded poorer r^2 values. Factors such as air temperature, NO_x (high levels at some sites)

23   and lower O_3 concentrations in Europe were suggested to account in part for the differences

24   between U.S. and Europe study results. Bermejo et al. (2002), in a study in Spain, improved the

25   model by comparing the biomass ratio of these white clover isolines to measures of O_3 uptake

26   (flux) rather than an exposure index (AOT40). Together, these studies indicate that systems

27   such as the white clover model can help reveal O_3 exposure-response relationships that can

28   provide valuable information regarding ambient O_3 conditions in a given location. Table 9-3

29   lists the advantages and disadvantages of the use of cultivar comparisons in assessing O_3 effects

30   of plants.

31

Table 9-3. 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
Preliminary tests to insure sensitivity and growth patterns of genotypes used are consistent are needed
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
Closely monitor plants for presence of other factors that may cause a misinterpretation of results

1   *Dendrochronological techniques*

2   It has been difficult to determine whether O$_3$ significantly affects tree growth and

3   productivity in the field, because O$_3$ concentrations are omnipresent and tree response to this

4   pollutant is altered by many factors. The use of dendrochronological techniques to answer

5   questions regarding ambient O$_3$ effects on forest growth and ecosystem function has recently

6   emerged as a very useful biomonitoring methodology (Cook, 1990; McLaughlin et al., 2002).

7   The technique is useful when either instrument or passive O$_3$ monitoring methods are used to

8   determine ambient O$_3$ conditions.

9   Initial experiments were primarily correlative in nature and attempted to relate symptoms

10   of visible injury with growth losses as revealed by tree ring analysis (Arbaugh et al., 1998;

11   Benoit et al., 1983; Peterson et al., 1995; Somers et al., 1998; Swank and Vose, 1990). These

12   studies evaluated radial growth patterns determined by cores removed from trees in the presence

13   or absence of overt O$_3$ injury symptoms.

14   The method has also been adapted to better understand forest ecosystem function

15   (McLaughlin and Downing, 1996; McLaughlin and Downing, 1995; Bartholomay et al., 1997;

16   McLaughlin et al., 2003). The response of mature loblolly pine (*Pinus Taeda*) growing in

1 eastern Tennessee to ambient O_3 and moisture stress was evaluated by McLaughlin and Downing

2 (1996, 1995) over a 6-year period (1988 to 1983). They made radial growth measurements

3 from 12 to 37 times per year using dendrometer bands and determined relationships between O_3,

4 moisture stress, and radial growth. Exposures to O_3 concentrations $\geq 0.04\ \mu l\ L^{-1}$ with high

5 temperatures and low soil moisture resulted in short-term depression in radial growth.

6 Reductions in growth were estimated to vary from 0 to 15% per year and averaged

7 approximately 5% per year.

8   Bartholomay et al. (1997) examined white pine (*Pinus strobus*) radial growth in eight

9 stands throughout Acadia National Park, Maine over a 10-year period from 1983 to 1992. They

10 related growth rates to several factors, including O_3 concentration. Ozone levels were negatively

11 correlated with radial growth in seven of the eight stands. Site characteristics were important in

12 the relationship: stands growing on shallow, poorly drained soils were most sensitive to O_3 in

13 the late portion of the growing season, possibly due to premature senescence of foliage.

14 However, litterfall measurements were not reported. Trees growing on better sites were more

15 sensitive to O_3 during the entire growing season, indicating the possibility of high O_3 uptake

16 rates throughout the growing season. Although these field studies (Bartholomay et al., 1997;

17 McLaughlin and Downing, 1996; McLaughlin and Downing, 1995) did not compare the firm O_3

18 effects on the two pine species, they indicate that potential interactions exist among O_3 and other

19 climatic and edaphic factors, such as temperature and soil moisture.

20   Using both automated and manual dendrometer bands, McLaughlin et al. (2002) examined

21 the growth response of yellow poplar (*Liriodendron tulipifera*) trees recently released from

22 competition. In addition to measuring growth, sap flow measurements were conducted and soil

23 moisture was measured in the vicinity of the trees. However, O_3 concentrations were low and

24 there were no negative O_3 effects observed in this 1-year study. Advantages and disadvantages

25 of dendrochronology techniques for evaluating whole-tree physiological responses for individual

26 trees and forest stands are listed in Table 9-4.

27   The use and evolution of various dendrochronological methods in the field of air pollution

28 effects research is reviewed in detail by McLaughlin et al. (2002). Automated dendrometer

29 bands provide a powerful tool for measuring radial growth responses of trees on an hourly or

30 daily basis. Diurnal patterns of growth can be related to water use and O_3 concentrations using

31

Table 9-4. 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 relate these to physiological function
Provide information on forest function related to ambient O$_3$ concentrations
Can link data with process level growth models

Disadvantages
Results are generally correlative in nature with no true control
Need background O$_3$ and meteorological data (historical records)
Need to account for other factors such as competition, in analyzing data
Individuals need to be trained in counting growth rings
Replication can be difficult (expensive and technological limitations)
Complicated statistical analyses are sometimes required
Can be expensive, especially if using automated growth (dendrometer) bands

1   time-series analyses. The major drawbacks of the method are that it is expensive and time

2   consuming.

3

4   **9.2.3.5   Calibrated Passive Monitors**

5   Many studies have used passive monitors in the mapping of ambient O$_3$ concentrations,

6   especially in remote areas (Cox and Malcolm, 1999; Grosjean et al., 1995; Krupa et al., 2001).

7   Since they are cumulative recording devices, they cannot reveal short-term variations in O$_3$

8   concentration but only the total exposure over a given interval, usually 7 days. Thus, they

9   produce a measurement that resembles the instrumentally derived exposure SUM00 index.

10   Runeckles and Bowen (2000) used the ZAPS system described in Section 9.2.2.3 to subject

11   both crops and passive monitors (Williams, 1994) to a range of exposures. Passive monitors

12   were also exposed at 16 agricultural field sites along a transect through the Fraser Valley, British

1   Columbia, Canada. Most field sites were downwind of the Greater Vancouver metropolitan

2   area. All passive monitors were replaced at weekly intervals and the data from those in the

3   ZAPS plots were "calibrated" to crop responses by means of Weibull exposure-response

4   functions. Since the meteorological conditions throughout the valley were reasonably consistent

5   from site to site, the use of these functions with data from the network passive monitors as inputs

6   permitted the estimation of crop losses at the network sites. The overall method was thus a

7   hybrid of several methodologies.

8   Although based on a single study, the use of passive monitors has potential for assessing

9   crop losses at sites removed from locations with known ambient O_3 concentrations. Provided

10   that the network and calibration sites have similar meteorological conditions, the method yields

11   crop loss estimates that are responses to local ambient O_3 levels as influenced by local

12   meteorological conditions

13

14   **9.2.4 Numerical/Statistical Methodologies**

15   Proper experimental design strategies including replication, randomization, and

16   experimental protocols are paramount in O_3-effects research. These have been discussed in detail

17   in previous O_3 AQCDs (U.S. Environmental Protection Agency, 1986, 1996), as have the

18   different statistical analytical procedures used to determine the probable significance of results.

19   However, new investigative approaches have demanded the adoption of new analytical methods.

20   For example, the use of dendrochronological techniques has led to the use of time-series analysis

21   (McLaughlin et al., 2003) and linear aggregate models (Cook, 1990), as reviewed by

22   McLaughlin et al. (2002).

23   In spite of the rigors of the analyses, many differences occur in the published literature for

24   almost any plant response to O_3 stress. Differences inevitably result from different researchers

25   studying different locations, using different experimental methodologies and genetically

26   different plant material even when using a common species. The techniques of meta-analysis

27   can be used to consolidate and extract a summary of significant responses from a selection of

28   such data.

29   Despite the differences in responses in the 53 primary studies used, a recent meta-analysis

30   by Morgan et al. (2003) of the effects of O_3 on photosynthesis, growth, and yield of soybean

31   (*Glycine max*) 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 exposure to ~70 ppb 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 (ANN) 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 9.2.3.3 (Ball et al., 1998). The multi-factor model for predicting the effects of ambient O_3 on 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, Jr., 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 Criteria Document (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

1   advantages and shortcomings of many different models and make the important point that most

2   of the models that have been described provide *consequence* assessments that quantify the

3   magnitudes of effects, but not *risk* assessments that quantify the likelihoods of such effects.

4   Descriptions of several specific models are provided in other sections of this criteria document,

5   and advantages and disadvantages of modeling techniques used in assessing O_3 effects on plants

6   are summarized in Table 9-5.

7

8

Table 9-5. Advantages and Disadvantages of Modeling Techniques Used in Assessment of O_3 Effects on Plants

Advantages
• Provides an understanding of cause-effect relationships over time

Disadvantages
• Have to make assumptions based on scarcity of data
• Most models are very complex and difficult to understand
• Need to be evaluated for predictive validity

9.2.5 Improved Methods for Defining Exposure

2   Ambient air quality is defined in terms of the measured O_3 concentrations in the air at some

3   standard elevation above ground level. Compilations of such concentration data have long been

4   used as surrogates of the exposures to which plants are subjected. However, as long ago as

5   1965, field research provided evidence that plant response was a function, not of ambient O_3

6   concentration *per se*, but of the estimated flux of O_3 to the plant canopy (Mukammal, 1965).

7   Subsequently, Runeckles (1974) introduced the term "effective dose" to define that part of the

8   ambient exposure that was taken up by a plant. Fowler and Cape (1982) later referred it as

9   "pollutant applied dose" (PAD), defined as the product of concentration, time and stomatal

10   (or canopy) conductance, with units g m^{-2}. Such estimates of O_3 uptake or flux provide a more

11   biologically relevant description of exposure than the simple product of concentration and time

1   alone, and formed the basis of Reich's 1983 "unifying theory" of plant response to O_3 (Reich,

2   1983).

3   However, it was not until the early 1990s that the inherent advantages of using O_3 flux

4   rather than O_3 concentration as a basis for determining response effects began to be widely

5   accepted, as demonstrated by the subsequent increase in publications involving flux

6   measurements and modeling (e.g., Fuhrer et al., 1997; Grünhage and Jäger, 2003; Grünhage

7   et al., 1993, 1997; Massman et al., 2000; Musselman and Massman, 1999; Pleijel, 1998). A key

8   requirement for flux determination is the measurement of stomatal or canopy conductances,

9   using established porometer/cuvette techniques or eddy correlation methods. The usefulness and

10   relevance of flux as a measure of exposure are discussed in detail in Section 9.5.

11   Efforts to develop models of O_3 deposition and stomatal uptake are currently under way

12   with a view to providing improved assessments of the risks to vegetation across Europe

13   (Emberson et al., 2000; Simpson et al., 2001; Simpson et al., 2003).

14

15

9.3   SPECIES RESPONSE/MODE-OF-ACTION

9.3.1   Introduction

18   The evaluation of O_3 risk to vegetation requires fundamental understanding of both the

19   functioning of the vegetation and how external environmental influences can alter that function.

20   For biological organisms subjected to atmospheric O_3, those alterations can be complex and

21   multiple. In addition, biological organisms have plasticity to external interactions due to their

22   complex internal, self-correcting systems, making the task of identifying their "correct"

23   functioning difficult. This section emphasizes reactions of O_3 with the cell and tissue rather than

24   the whole plant to describe the fundamental mechanisms known to govern the response of the

25   plant to O_3 exposure.

26   The many regulatory systems contained in leaves change both as a function of leaf

27   development and in response to various environmental stresses. Leaves function as the major

28   regulators of anatomical and morphological development of the shoot and control the allocation

29   of carbohydrates to the whole plant (Dickson and Isebrands, 1991). This section discusses the

30   movement of O_3 into plant leaves and their biochemical and physiological responses to O_3.

1   The 1996 criteria document (U.S. Environmental Protection Agency, 1996) assessed the
2   information available at that time concerning the biochemical and physiological responses to the
3   movement of O_3 into plant leaves. This information continues to be valid. Ozone uptake in a
4   plant canopy is a complex process involving adsorption to surfaces (leaves, stems, and soil) and
5   absorption into leaves (Figure 9-1). However, the initial biochemical changes that result within
6   leaf cells after the entry of O_3 and how these changes interact to produce plant responses remain
7   unclear. The response of vascular plants to O_3 may be viewed as the culmination of a sequence
8   of physical, biochemical, and physiological events. Only the O_3 that diffuses into a plant
9   through the stomata (which exert some control on O_3 uptake) to the active sites within a leaf
10   impairs plant processes or performance. An effect will occur only if sufficient amounts of O_3
11   reach sensitive cellular sites that are subject to the various physiological and biochemical
12   controls within the leaf cells. Ozone injury will not occur if (1) the rate and amount of O_3 uptake
13   is small enough for the plant to detoxify or metabolize O_3 or its metabolites, or if (2) the plant is
14   able to repair or compensate for the O_3 impacts (Tingey and Taylor, 1982; U.S. Environmental
15   Protection Agency, 1996). Therefore, a precondition for O_3 to affect plant function is that it
16   must enter the stomata and be absorbed into the water lining the mesophyll cell walls. The
17   response of each plant is determined by the amount of O_3 entering the leaves, which varies from
18   leaf to leaf.
19   Some potentially significant processes have been investigated since the 1996 criteria
20   document, especially detoxification and compensatory processes. The role of detoxification in
21   providing a level of resistance to O_3 has been investigated; however, it is still not clear as to what
22   extent detoxification can protect against O_3 injury. Data are needed especially on the potential
23   rates of antioxidant production and on the subcellular localization of the antioxidants. Potential
24   rates of antioxidant production are needed to assess whether they are sufficient to detoxify the O_3
25   as it enters the cell. The subcellular location(s) are needed to assess whether the antioxidants are
26   in cell wall or plasmalemma locations that permit contact with the O_3 before it has a chance to
27   damage subcellular systems. Various forms of compensation, especially the stimulation of new
28   leaf production and of higher photosynthetic performance of new leaves, have been reported.
29   Although these processes divert resources away from other sinks, these forms of compensation
30   may counteract the reduction in canopy carbon fixation caused by O_3. The quantitative
31   importance of these processes requires investigation.
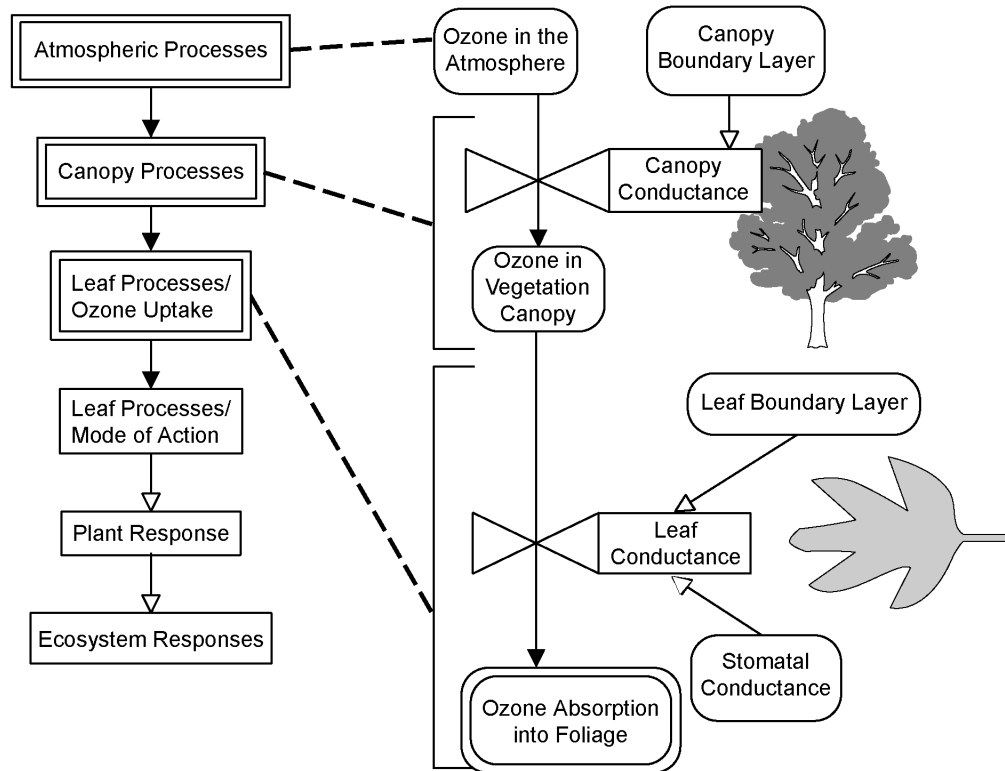
Figure 9-1. **Ozone uptake from the atmosphere. Ozone moves from the atmosphere above the canopy boundary layer into the canopy primarily by turbulent air flow. Canopy conductance, controlled by the complexity of the canopy architecture, is a measure of the ease with which gases move into the canopy. Within the canopy, O_3 is adsorbed onto surfaces as well as being absorbed into the foliage. Foliage absorption is controlled by two conductances, leaf boundary layer and stomatal, which together determine leaf conductance. The solid black arrows denote O_3 flow; dotted arrows indicate processes affecting uptake or response to O_3. Boxes at the left with double borders are those processes described in the figure.**

1   As a result of the research since the 1996 criteria document (U.S. Environmental Protection
2   Agency, 1996), the way in which O_3 exposure reduces photosynthesis, especially its effects on
3   the central carboxylating enzyme, Rubisco (ribulose-1,6-P_2-carboxylase/oxygenase1), is better
4   understood. The rate of leaf senescence has been shown to increase as a function of increasing
5   O_3 exposure. The mechanism of the increased senescence is not known, and, hence, it deserves
6   further study.

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

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

13   This section focuses on reactions of O_3 within cells and cellular tissue, in order to explain
14   known mechanisms that govern plant responses. The processes that occur at cell and tissue
15   levels within the leaf will be divided into several steps beginning with O_3 uptake and its initial
16   chemical transformations into a series of currently unknown, but suspected toxic, chemicals
17   (Figure 9-2). The discussion will then focus sequentially upon various cell regions, their general
18   physiology, and the changes that may occur within a plant after O_3 exposure. This is important
19   because the varying responses of the different plant species in a community ultimately lead to
20   an ecosystem response. Finally, a general summary is presented that discusses the known or
21   suspected changes that occur within the whole plant.

22

9.3.2   Mechanisms of Ozone-Induced Plant Alterations

24   Plant adaptations for surviving O_3 stress include exclusion or tolerance of it or its products
25   (Levitt, 1972; Tingey and Taylor, 1982). Ozone may be excluded from tissues or cells via
26   stomatal closure, by extracellular oxidants, or by membrane impermeability to O_3 or its products.
27   Past investigations of O_3 injury have indicated that physiological and metabolic changes occur
28   (see Heath, 1998; Reddy et al., 1993; Harris and Bailey-Serres, 1994). Many of these changes
29   are likely initiated via gene expression. During the last decade, our understanding of the cellular
30   processes within plants has increased. Although the fundamental hypotheses concerning
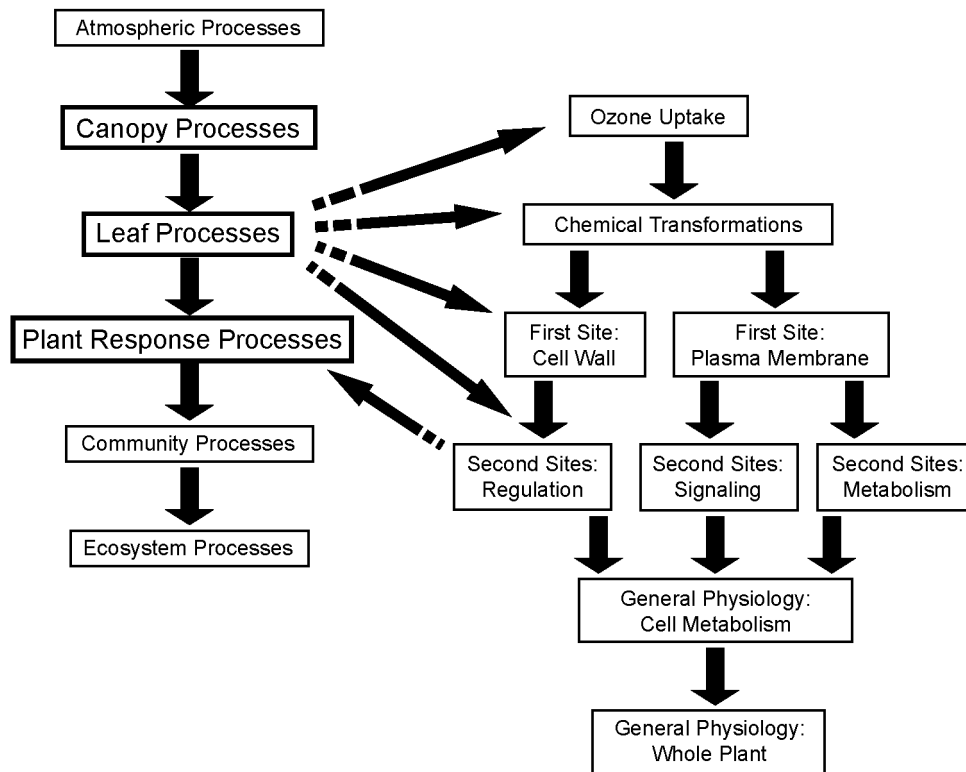
Figure 9-2. Absorption and transformation of O$_3$ within the leaf. The varied processes are broken down in to smaller mechanistic steps that lead from uptake of atmospheric O$_3$ into the alterations which may occur within the individual plant. Each plant responds to the O$_3$ level and therefore interacts with the total ecological setting to generate an ecosystem response due to the O$_3$.

1   O$_3$-induced changes in physiology have not changed, a more complete development of the

2   theories is now nearing possibility.

3

4   **9.3.2.1 Changes in Metabolic Processes**: **Current Theories**

5   The current hypotheses regarding the biochemical response to O$_3$ fumigation revolve about

6   injury and its prevention. These are well discussed by Pell et al. (1997) and are listed below in

7   no order of importance. Although they are listed separately, some may be interlinked and related

8   to each other.

9

1   (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   (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 the sufficient abundance. However, oxidant entry that occurs rapidly can overwhelm the antioxidant response.

3   (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   (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   (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   (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 what enzymes and metabolites could be assayed were. Thus, these results were based upon what could be done, rather than a coherent hypothesis. The best examples are listed in Dugger and Ting (1970).

7   The latter two theories can be restated as a loss of productivity with three possible

8   somewhat-independent causes — (a) a reduced production of the basic building blocks of growth

9   and, hence, a slowing of growth in at least one organ; (b) a reduced ability to reproduce, leading

10   to a decreased production of viable seeds or of fruits and nuts; and (c) a decreased ability to

11   mount a defense against pathogens or insects, leading to weaker plants, which are more liable to

12   be overcome by other stresses. It is important to separate out effects that may be detrimental or

1   disfiguring, such as the production of visible injury, but which have not been shown to lead

2   directly to a loss of productivity due to possible compensation by the remaining tissue. The loss

3   of some localized regions of photosynthesis tissue has not been shown to lead directly to a loss

4   of productivity.

5

6   **9.3.2.2   Modifications of Plant Physiological Processes**

7   The discussion that follows will focus on physiological processes; the species used to

8   develop an understanding of these processes are relatively unimportant. The study of any plant

9   that can help increase the biological understanding of the response of plants to O_3 exposures is of

10 use, regardless of how sensitive it is to O_3. Therefore, *Arabidopsis*, whose physiology and

11 genome continue to be studied and described by a large number of scientists is an appropriate

12 plant for studying O_3 injury. Though the responses of mature trees and understory plants are

13 critical to understanding plant interactions at an ecosystem level, the time required for trees to

14 reach maturity makes using them to study biological mechanisms a poor choice.

15 The high levels of O_3 used for some investigations do not automatically invalidate the

16 results obtained in those studies. Typically when a new hypothesis is being investigated,

17 extreme levels of the toxicant are used to clearly determine its effects. The older studies that

18 used concentrations as high 1 ppm, an extreme level, helped to define current studies. Later

19 experiments have used concentrations nearer ambient levels. Many of the current studies on

20 physiology use exposures between 0.15 and 0.25 ppm, which though higher than ambient levels

21 in some areas of the country, bypass confounding changes but allow for rapid experiments.

22 Three forms of air pollutant-induced injury patterns currently are known to exist: (a) acute

23 stress, generated by high atmospheric concentrations of pollutants for short periods of time;

24 (b) chronic stress, generated by lower concentrations of pollutants for long periods of time; and

25 (c) accelerated senescence, generated by very low concentrations of pollutants for very long

26 periods of time. At higher levels, distinct visible injury generally occurs due to cellular and

27 tissue death of regions of leaf mesophyll cells. This leads to a decline in the total area of

28 metabolically active tissue, with consequent loss of membrane integrity, loss of metabolites into

29 the extracellular tissue space, and formation of oxidative products. When no visible injury is

30 observed, lowered rates of photosynthesis or productivity are often used to index injury. Under

31 these conditions, metabolism is altered and the pool sizes of many metabolites are changed.

1   More importantly, the altered biochemical states within the tissue lead to the inability of the

2   plant to respond properly to existing environmental conditions and to other stressors (Manning

3   and Keane, 1988; Heath, 1988; 1994b; Koziol and Whatley, 1984; Schulte-Hosted et al., 1988).

4

5   ### 9.3.3 Ozone Uptake by Leaves

6   Plants respond to O_3 similarly to other stressors on several levels: exclusion, tolerance, and

7   repair (Levitt, 1972; Tingey and Taylor, 1982). The response mechanism depends upon the O_3

8   concentration, environmental conditions, and the developmental and metabolic state of the plant

9   (Guzy and Heath, 1993). These responses are detrimental to plant productivity because they cost

10 the plant metabolic resources. In some cases, the stomata close under the O_3 exposure,

11 excluding the pollutant from the leaf interior and preventing injury. However, if this happens

12 too often, CO_2 fixation is also inhibited and plant productivity suffers.

13 Atmospheric O_3 does not cause injury, but rather it is the O_3 that enters the plant that

14 causes an effect (Tingey and Taylor, 1982). Three well-defined, sequential processes control the

15 movement of O_3 from the atmosphere into the sites of action within the leaf and must occur to

16 trigger O_3 stress (Heath, 1980). The processes are: (1) entry of O_3 into the leaf, (2) reactions of

17 O_3 and its possible reaction product(s) in the water phase at cell surfaces, and (3) movement of

18 O_3 reaction product(s) into the cell with enzymatic or chemical transformation of those products

19 in the cell.

20

21 *Process 1*. *Entry of* O_3 *into the leaf.* Often incorrectly, the external concentration of O_3 is

22 used to give an indication of "dose" (Heath, 1994a). Ozone-induced changes on the plants

23 cuticle are minimal, and O_3 does not penetrate the cuticle (Kerstiens and Lendzian, 1989) to

24 cause an effect. As O_3 has no easily measured isotope, virtually no measurements have been

25 done on an actual dose of O_3, i.e., the amount of O_3 which reacts with individual biochemicals in

26 the leaf. Yet the measurement of dose will be the amount of O_3 expected to penetrate into the

27 tissue through the stomata. Dose is expressed as a rate of delivery to a surface area ($mol/m^2 \, s^{-1}$).

28 Whether dose or total accumulation ($mole/m^2$, rate integrated over exposure time) is most critical

29 for the development of injury remains a major question.

30 Ozone uptake includes gaseous diffusion through the leaf boundary layer and stomata into

31 the substomatal cavity (Figure 9-3). Although the movement of pollutants through a boundary
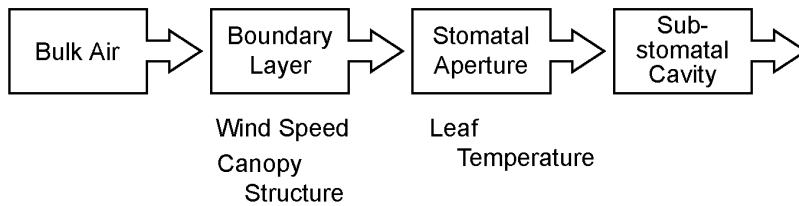
Figure 9-3. **The uptake of O_3 into the leaf. Each of the individual concentration layers of O_3 represents a different process of movement and of plant/ microenvironmental interaction. This figure leads into Table 1, in which the amounts of O_3 along the pathway are calculated.**

1   layer into the stomata region is known to be important, and even rate limiting in many cases of

2   low wind velocity, its description has been defined from aeronautical concepts and usually

3   relates to smooth surfaces that are not typical of leaf-surface morphology; however, it is nearly

4   the only treatment available (Gates, 1968). Once through the boundary layer, the gas must enter

5   the leaf through the stomata. The entry of gases into a leaf is dependent upon the physical and

6   chemical processes of gas phase and surfaces and is a well defined path that approximately

7   follows a linear flux law of:

8

$$j = g(C_o - C_i) \tag{9-1}$$

9

10   where the flux, j, into the internal space of a leaf is related to the conductance, g, through the

11   boundary layer and stomata and the gradient of concentration of gas from the outside, C_o,

12   inwards, C_i. This formulation has been used for years for both water and CO_2 (Figure 9-4), and

13   for regions of varied CO_2 concentration that correspond to C_o (CO_2 of the atmospheric air, below

14   the leaf proper) and C_i (CO_2 near the leaf's spongy mesophyll cells) (Farquar and Sharkey, 1982;

15   Ball, 1987).

16   In the past, the internal concentration of O_3 has been assumed to be zero (Laisk et al.,

17   1989), due to early studies that found that virtually no O_3 could pass through a leaf. That was

18   expected because O_3 is extremely reactive with cellular biochemicals. If the assumption that the
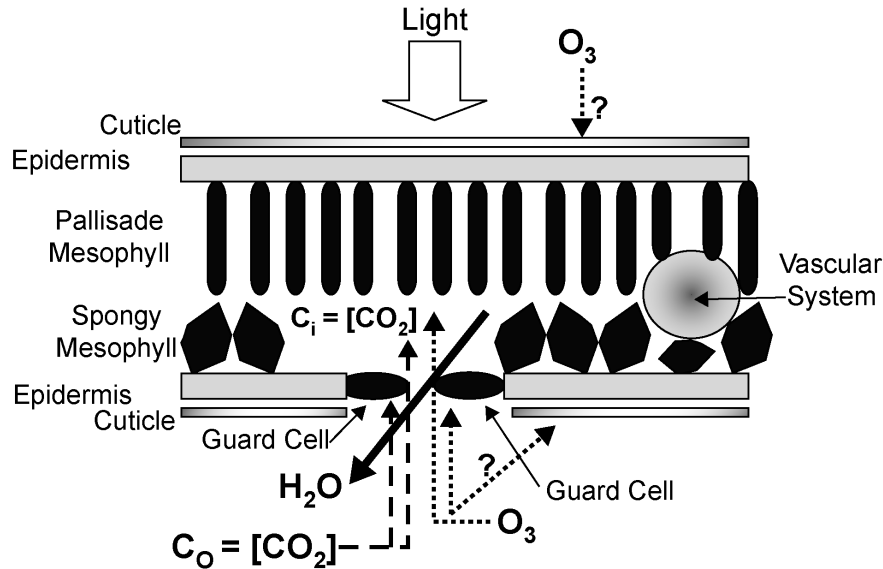
Figure 9-4. **The microarchitecture of a dicot leaf. While details among species vary, the general overview remains the same. Light that drives photosynthesis generally falls upon the upper (adaxial) leaf surface. Carbon dioxide and O_3 enters through the stomata on the lower (abaxial) leaf surface, while water vapor exits through the stomata (transpiration).**

1   internal concentration zero is correct, then the effective delivery rate for O_3 is given as $g \times C_o$,

2   with stomatal conductance being the major regulatory control (Taylor, Jr. et al., 1982; Amiro

3   et al., 1984). However, a recent study by Moldau and Bichele (2002) indicated that the internal

4   O_3 concentration may not be zero, as previous assumed. Moldau and Bichele (2002) permitted

5   leaves of *Phaseolus vulgaris* L., which have stomata on both upper and lower leaf surfaces, to

6   take up O_3 at a high rate for 3 to 5 min. Exposure of the lower leaf surface resulted in up to 5%

7   of the O_3 that was taken up to be diffused through the leaf, emerging from the stomata on the

8   upper surface. This suggested above-zero concentrations of O_3 in the intercellular leaf air

9   spaces. The descriptive calculations and plots of Moldau and Bichele (2002) indicate that the

10   rise in internal O_3 level (for both concentrations of external O_3) within the first few minutes of

11   exposure is due to its reaction with an antioxidant, most probably absorbate, within the

12   apoplastic space of the leaf (Figure 9-5). The rate of rise is probably due to more complete

13   penetration of O_3 with a concurrent depletion of the external antioxidant. The rise peaks at about

14   2 min for 0.88 ppm and 3 min for 0.34 ppm and then falls to a lower level. This may be due to a
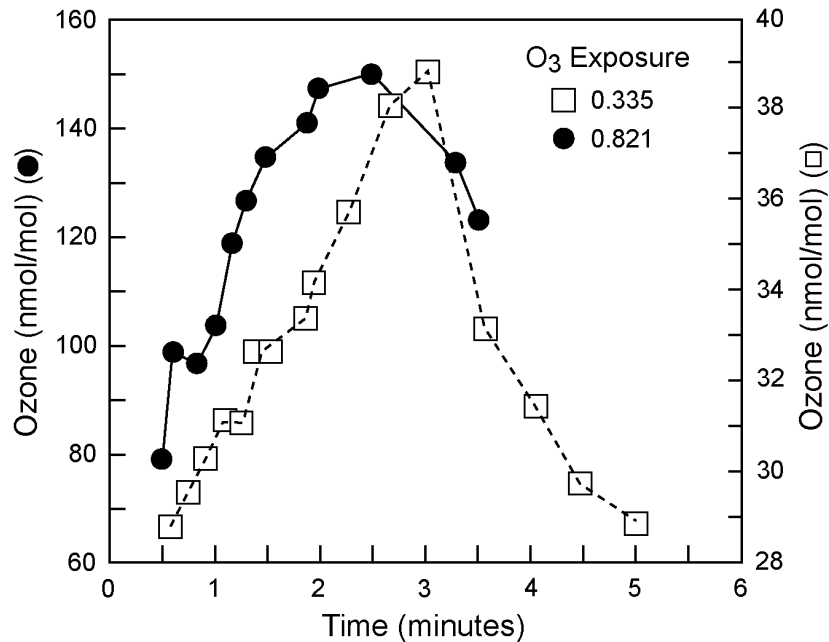
Figure 9-5. **The change in the O$_3$ concentration inside a leaf with time. Data are from O$_3$ exposures at two different concentrations.**

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

1   replenishment of the antioxidant. The authors saw no injury to the plasmalemma (as measured

2   by penetration of a dye) and no change in the stomatal conductance for the lower concentration

3   of O$_3$ (Moldan and Bichele, 2002). The higher level (0.88 ppm) caused the plasmalemma of the

4   mesophyll cells to pass a dye and a slight decline in stomatal conductance resulted at about

5   2.5 minutes. These data suggest that the antioxidant hypothesis is correct.

6   Gaseous pollutants flow from the substomatal cavity within the leaf into the cell, through

7   the cell wall. It is suspected that the internal concentration of the pollutant is not uniform within

8   the cavity. From within the wall, an equilibrium between the gas and aqueous phase must occur

9   at the interface where the gaseous species dissolve into the water according to Henry's Law

10   (Heath, 1980, 1987; Wellburn, 1990). It is important to understand exactly how much O$_3$ could

11   move into the tissue of the leaves. Calculations in Table 9-6 give an indication of the amount of

12   O$_3$ which may end up near the surface of cells within the leaf. The calculation is done for a

13   standard temperature (25 °C), an ambient concentration of O$_3$ (0.10 ppm), and for nonspecific

Table 9-6. 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:	$O_{3a} = 0.1$ 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 ($V_o = 22400$ m^3) will be used, from the perfect gas law with $R = 8.3144$	
Thus, the concentration of O_3 within the atmosphere is: $$C_{O_3} = \frac{O_{3a} \times 10^{-6} \cdot (T_a + 273.18)}{V_0 \times 273.18}$$	$C_{O_3} = 4.873 \times 10^{-12}$ 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:	$gs_{wv} = 1$ cm/s
The amount of O_3 that will penetrate inside the leaf (for a typical concentration of nearly zero inside the leaf), is: $$O_{3_L} = \sqrt{\frac{18}{48}} \frac{gs_{wv}}{100} C_{O_3}$$	$O_{3L} = 2.984 \times 10^{-14}$ 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:	$FW_L = 30$ mg/cm^2
Thus, the square surface area of the leaf will translate into water space (for concentration of chemicals), as: $$Ar_L = \frac{FW_L \times 0.85}{100}$$	$Ar_L = 0.255$ 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). $$O_{3_{Lc}} = (O_{3_L} / Ar_L) \times 3600$$	$O_{3Lc} = 4.21 \times 10^{-10}$ mol/(L hr)

Table 9-6 (cont'd). The Flow of Ozone into a Leaf and Possible Reactions

Thus, the maximum amount of toxic chemicals generated per hour in a leaf would be:	$O_{3Lc} = 0.42$ nmol/(L hr)

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

1   leaves. For example, 0.3 ppm would be the same general numbers but multiplied by 3.

2   Similarly for more closed stomata, the value of 1.0 cm/s (equivalent to about 400 μmole^{-2}-leaf

3   area s^{-1}) for a conductance would be reduced and the smaller values would lead to a smaller

4   amount of O_3 moving into the tissue. Nonetheless, these values give some indication of what

5   sort of chemical concentration can be expected. Under these conditions, a delivery rate of O_3

6   into the substomatal cavity near the spongy mesophyll tissue of about 0.42 nmol/(L hr) appears

7   to be reasonable.

8

9   *Process 2*. Ozone diffuses into the leaf air spaces and reacts either with varied biochemical

10   compounds (path 1) which are exposed to the air or is solubilized into the water lining the cell

11   wall of the air spaces (path 2). As shown in Figure 9-6, each reaction has the possibility of

12   transforming O_3 into another chemical species (a toxicant) which, in turn, may react with other

13   chemical species and lead to a cascade of reactions.

14   Within the stomata, gases react with the water at the cell's surface and generate new

15   species with the components within the cell wall region. The possible varied pathways are

16   depicted in Figure 9-7. Although these chemical reaction are poorly understood, some of the

17   fundamentals are known (Heath, 1987; 1988; Wellburn, 1990). Ozone reacts with organic

18   molecules at the double bonds to form carbonyl groups and, under certain circumstances,

19   generates peroxides, such as hydrogen peroxides (H_2O_2), superoxide (O_2^-) and its protonated

20   form ($HO_2^{\bullet}$), hydroxyl radical ($HO^{\bullet}$), and peroxy radical ($HO_2^{\bullet}$). Other chemicals present in the

21   water phase can lead to many other oxygenated moities (Figure 9-6). Each of the steps are

22   generally pH dependent (Walcek et al.,1997; Jans and Hoigne, 2000).

23
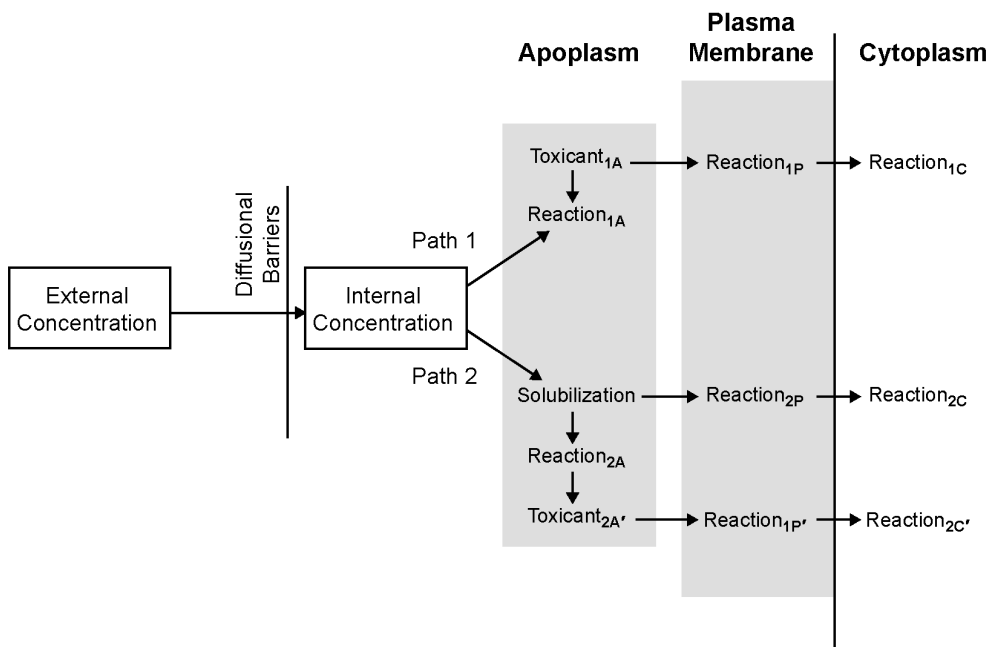
Figure 9-6. Possible transformations of O₃ within a leaf.
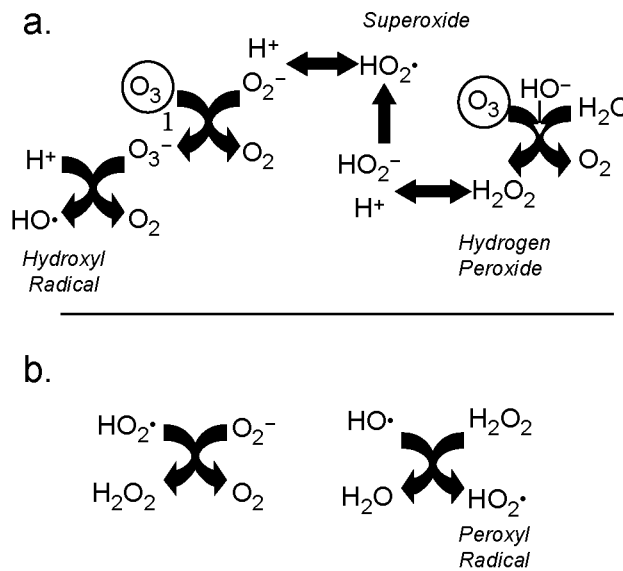
Figure 9-7. Possible reactions of O₃ within water. (a) Ozone reacts at the double bonds to form carbonyl groups. (b) Under certain circumstances, peroxides are generated.

1   Sulfhydryls are particularly easy targets, with the formation of disulfide bridges or sulfones

2   (Mudd and Kozlowski, 1975). In water, the reactions become more confusing, but some

3   products have been described by Heath and Castillo (Heath and Castillo, 1987), such as H_2O_2,

4    $HO^{\bullet}$, and O_2^{-} (Figure 9-7). Effective detoxification reactions can occur here via antioxidant

5   metabolites and enzymes, such as ascorbate, glutathione (GSH), and superoxide dismutase

6   (SOD), if they are present at high enough concentrations (Matters and Scandalios., 1987;

7   Castillo et al., 1987; Fong and Naider, 2002). If the levels are low, it is believed that stimulation

8   of their production is a response to O_3, albeit a slow one (Harris and Bailey-Serres, 1994).

9   Certainly it is possible that chemical modification of wall-specific biochemicals (Castillo et al.,

10   1987), such as glucan synthase (Ordin et al., 1969) and diamine oxidase (Peters et al., 1988), is

11   possible.

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

13   *transformations within the cell.* It is believed that the initial site of O_3 injury is near and within

14   the plasma membrane. Certainly, membrane functions, such as membrane fluidity (Pauls and

15   Thompson, 1980), permeability (Elkiey and Ormrod, 1979), K^{+}-exchange via ATPase reactions

16   (Dominy and Heath, 1985) and Ca^{2+} exclusion (Castillo and Heath, 1990), are changed. The

17   similarity of wounding responses (Langebartels et al., 1991) and O_3-induced membrane

18   disruption suggests the induction of normal wound-regulated genes (Mehlhorn et al., 1991;

19   Sandermann, Jr., 1998). This implies that O_3 can react with components of the cell wall

20   connected to the cytoplasm through the cell wall and membrane by membrane-specific proteins

21   not directly linked to transport.

22   Ozone is soluble in water and once having entered the aqueous phase, it can be rapidly

23   altered to form oxidative products that can diffuse more readily into and through the cell and

24   react with many biochemicals. Again, the presence of internal antioxidant would be critical to

25   reduce the concentration of most oxidants. A toxic product of O_3 may migrate through the

26   cytoplast to react with photosynthetic processes, or a spurious signal generated at the membrane

27   may affect some control process or signal transduction pathway (Schraudner et al., 1998;

28   Overmyer et al., 2000, 2003; Rao et al., 2000a, 2002; Sandermann, 2000; Rao and Davis, 2001;

29   Leitao et al., 2003; Vahala et al., 2003; Booker et al., 2004).

30

31

9.3.3.1 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 which will generate H_2O_2, $HO^\bullet$, and SO_2^-, as well as charged O_3 intermediates are indicated in Figure 9-8. Many of these complex reactions have been studied within water solutions through research of O_3 induced water purification and are very dependent upon solutes present with the solutions, including H^+ (see von Gunten (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 9-8, can be calculated from the reaction coefficient as given by Atkinson (1990) (Table 9-7). The double bond of the ascorbate molecule is particularly sensitive to O_3 attack. An unstable ozonide product is formed and then accelerates the breakage of the double bond, leading to the formation of two products because of the ring formation of the ascorbate molecule. These products are relatively unstable and can lead to further reactions not shown in Figure 9-8. The rates of reactions can be calculated (Heath, 1987) from the concentrations of O_3 that should occur within the wall regions (Table 9-6). 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 9-7 and, although some of the products would be formed through the Crigee Mechanism (see Figure 9-8a), they would be low in concentration[2]. While other radicals, such as hydroxyl radical (see Figure 9-8b) can attack double bonds, the products differ. Of particular note for later discussion, is the reaction of O_3 with ascorbate (see Mudd, 1996; Figure 9-8b), 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) calculates that O_3 has a half-life of about 7×10^{-8} s in a bilayer.

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[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.

[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.

a.

1. O_3 + $H_2C = CH_2$ $\xrightarrow{\text{Crigee Mechanism}}$ [Crigee intermediate structure] $\longrightarrow$ $\left[\begin{array}{c} H \\ HC=O \\ \\ O \\ \parallel \\ HC\text{-}OH \end{array}\right]$

2. $OH\bullet$ + $H_2C = CH_2$ $\longrightarrow$ $H_2\overset{OH}{\underset{|}{C}} - \overset{\bullet}{C}H_2$

3. $NO_3\bullet$ + $H_2C = CH_2$ $\longrightarrow$ $H_2\overset{ONO_2}{\underset{\backslash}{C}} - \overset{\bullet}{C}H_2$

b.
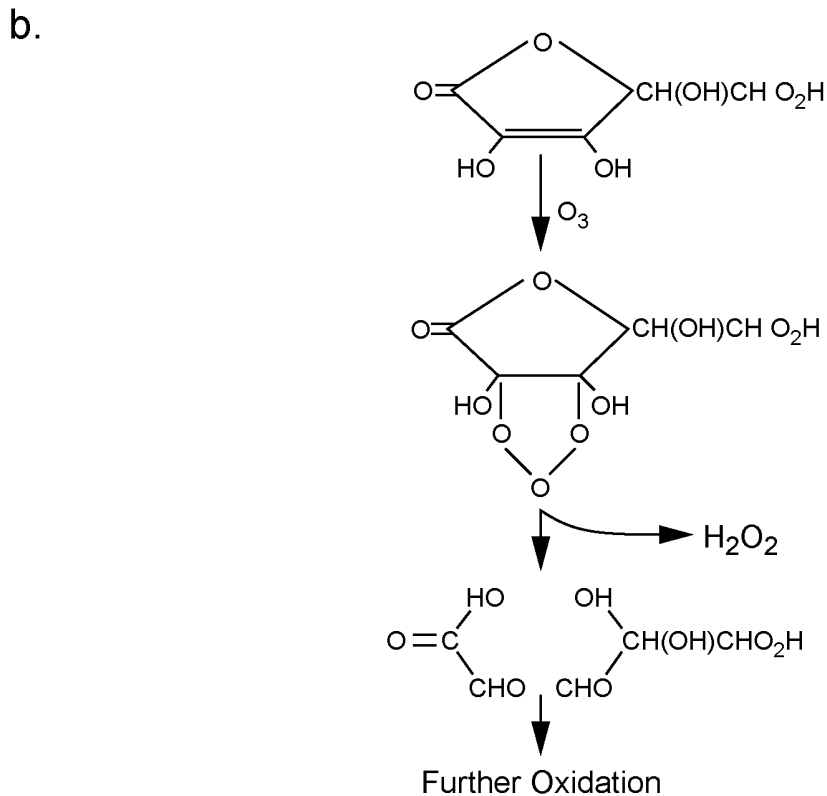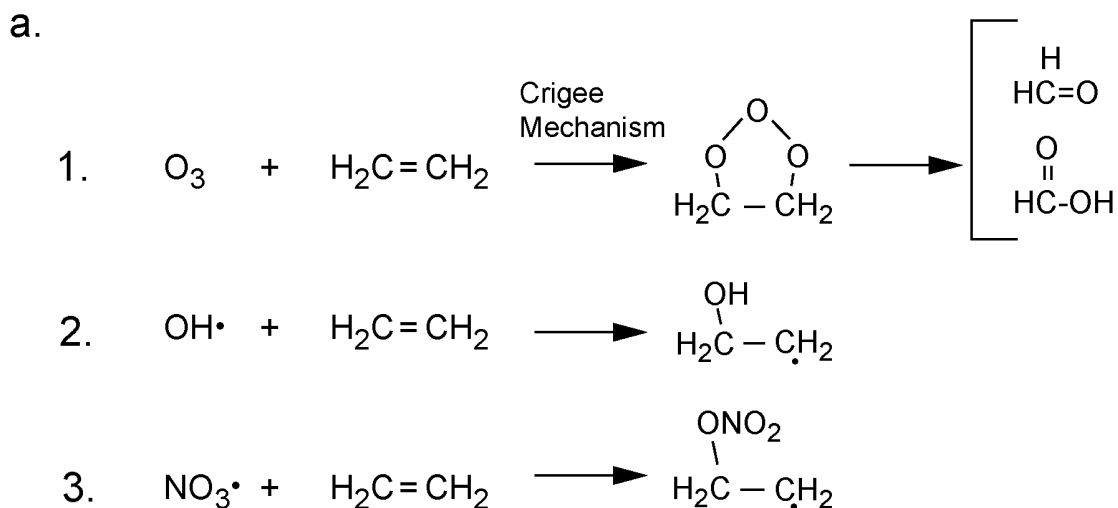
Figure 9-8a,b.   The Crigee mechanism of O_3 attack of a double bond. (a) The typical Crigee mechanism is shown in which several reactions paths from the initial product is shown. (b) Typical reaction of ascorbic acid with O_3.

Source: Adapted from Mudd (1996).

Table 9-7. 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 9-6).

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) $O_3 + O_2^- \rightarrow HO^\cdot + 2\,O_2 + OH^-$	$k_2 = 1.26 \times 10^9$ mole^{-1} L s^{-1}
(3) $O_3 + HO_2^- \rightarrow HO^\cdot + O_2^- + O_2$	$k_3 = 2.09 \times 10^6$ 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

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

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

1   However, the transit time through the lung lining fluid layer is about 2×10^{-6} s based upon a
2   reasonable estimate for the diffusion of O_3. This means that O_3 would suffer nearly 29 half-
3   lives[3] in passage through the layer, which would reduce O_3 to about 3×10^{-9} of the original
4   concentration — zero for all practical aspects. In the same publication, Pryor points out that any
5   sulfhydryl or ascorbate would interact strongly with O_3, further reducing its net concentration.

---

[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.

1   The reactivity of cysteine is 10^9, while the reactivity of tryptophan, methionine, polyunsaturated

2   fatty acids, and tyrosine is about 2×10^6, and that of phenylalanine is only 10^3. These numbers

3   are similar to what has been found for O_3 reactivity with amino acids and proteins in aqueous

4   solutions. In glycophorin (Banerjee and Mudd, 1992) and cytochrome C (Mudd et al., 1996,

5   1997b) in aqueous solutions, only the methionine was oxidized by O_3, producing sulfoxide.

6   In other proteins lacking methionine, tryptophans were oxidized only if they were in an exposed

7   position on the surface of the proteins (Mudd et al., 1997b). Treatment of red blood cell ghosts

8   with O_3, oxidized peripheral proteins of the plasma membrane before it oxidized lipids (Mudd

9   et al., 1997a).

10

11   **9.3.3.2   Toxicants Within the Wall Space**

12   While Mehlhorn et al. (1990) is often thought to have shown that free radicals were formed

13   in plant leaves under O_3 exposure, careful reading of that paper clearly shows that there was no

14   real evidence of free radicals induced by O_3. Living tissues have many free radical signals,

15   making it difficult to observe changes in free radicals. Further, the work of Grimes et al. (1983)

16   has also been cited as showing the presence of free radicals in living tissues due to O_3 exposure;

17   however, no radical signals were found unless certain organic acids (e.g., caffeic acid) were

18   added to the tissue with the O_3 exposure. They used the radical trap TMPO (tetramethylphrrolise

19   1-oxide) which reacts with many types of free radicals to form a stable radical that can be used

20   to "trap" or increase the amount of radical present (see Figure 9-9a). Ozone would directly react

21   with this trap only if it were bubbled into the solution, not passed over the top of the solution.

22   In the presence of sorbitol or caffeic acid, the trap would indicate the presence of OH radical,

23   which would mean that $O_3 \rightarrow HO^{\bullet}$. Superoxide dismutase, catalase, or EDU had no effect upon

24   this signal, suggesting O_2^- and H_2O_2 were not involved in the above sequence. Both O_3 and O_3

25   plus caffeic acid had no effect upon the protoplasts' intactness or viability. Thus, 10^{-5} M $HO^{\bullet}$

26   and/or 0.30 to 0.40 ppm O_3 did not react with the cell membrane. They found no signal in

27   normal cells after subjecting the leaf to O_3 and concluded that the radicals were produced via a

28   concerted mechanism with the acid. This does not fit with the mechanism postulated by

29   Mehlhorn et al. (1991), which involved a reaction of wound-induced ethylene and O_3 at the wall

30   level to generate some free radicals.

31

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 9-9. **Varied ESR radicals, trapped and not, generated by ozone 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 0.10 ppm O_3 for 4 hours. The lower trace is the ESR signal produced with 0.3 ppm O_3 (Mehlhorn et al., 1990). (c) The EPR signal produced within a bluegrass leaf exposed to ppm of O_3 for 1 h (Runeckles, 1997). Although no trapping agent was used in this experiment, the signal is complex because of various free radicals normally present within the illuminated leaf.**

1 The hypothesis that the production of wound-induced ethylene by O_3 exposure and its

2 reaction with O_3 would result in the production of radicals was tested by Mehlhorn et al. (1990),

3 using electron paramagnetic resonance spectroscopy. After 4 h of 300 ppb, an EPR signal of a

4 compound was detected which resembled a butonyl radical (Figure 9-9b) . Using 70 ppb, the

5 signal was reduced by about one third of an ethyl radical[4], leading to injury. However, the

6 spraying of the plant with 1-aminoethoxyvinyl-Gly (AVG), which reduces the production of

---

[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 $= 1.02 \times 10^3$ M^{-1} s^{-1} (Atkinson R., 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.

1   ethylene and visible injury, had no effect upon the EPR signal, suggesting that the radical is not

2   on a direct sequela to visible injury.

3   Runeckles and Vaartnou (1997; Figure 9-9c) discovered a signal by subtracting other EPR

4   signals of the leaf, which seemed to be due to an O_3 reaction with plant material, using 0.48 ppm

5   O_3. This difference signal looked very much like O_2^-. At a lower concentration, they observed

6   that this signal still occurred but accumulated more slowly. Both bluegrass and ryegrass leaves

7   seemed to saturate after about 5 h of exposure at 22 to 28 units of signal, while radish leaves

8   reached a maximum of 7 units at 3 h and then declined. The problem, which is typical of any of

9   these methods, was that the detached leaf had to be rolled and placed into the EPR detection

10   cavity. Reichenauer et al. (1998) also detected an undefined free radical signal that seemed to be

11   related to a Mn(II) spectrum. The Nandu and Perlo cultivars of wheat were more sensitive to O_3

12   than Extradur (according to growth rate and closure of stomata under an O_3 exposure of 80 ppb

13   for 8 h/day, 7 days/week over 100 days), and these more sensitive cultivars had a greater, but

14   insignificant (P = 15%) EPR signal. Thus, data showing any production of a free radical must be

15   approached with some skepticism.

16   With an O_3 delivery rate of about 25 μM/h (Table 9-6), only 250 μM would be found after

17   a full day, if all of the O_3 were stable. While the use of free radical traps is the best method

18   available to observe any build-up of radicals, the traps are not as specific to individual radicals.

19   Currently, studies should be looking for hydroxyl radicals, superoxide, hyroperoxides, ethylene

20   radicals, and ascorbate radicals.

21

22   **9.3.3.3 Products of Ozone**

23   Ozone should reach a certain concentration in the substomatal cavity, which is dependent

24   upon its entry speed and its reactivity with the wall constituents. Once near the apoplastic space,

25   O_3 moves in two different pathways (Figure 9-6). It can react with constituents that are within

26   the wall as a gas in reaction 1A (path 1); or it can solubilize into a water space and travel to

27   another region within the water space and react through reaction 2A (path 2).

28

29   ***Hydrogen Peroxide***

30   Hydrogen peroxide, until recently was thought to be purely a toxic compound for cells.

31   However, it is now clear that it functions as a signaling molecule in plants and mediates

1   responses to abiotic and biotic stressors (Figure 9-10). Generation of H_2O_2 is increased in

2   response to various stressors, implicating it as key factor mediating the phenomena of

3   acclimation and cross tolerance, in which exposure to one stressor can induce tolerance of

4   subsequent exposure to the same or different stresses (Neill et al., 2002). The signaling response

5   to attack by invading pathogens using H_2O_2 has been described (Mehdy, 1994; Simon-Plas et al.,

6   1997). The reactions leading to hypersensitive cell death are caused by a pathogen recognition

7   step (Figure 9-10a), probably due to the plant cell wall releasing oligosaccharides in response to

8   the pathogen enzymatically breaking down the cell wall to penetrate it. A feed-forward step in

9   which H_2O_2 increases the level of benzoic acid leads to the activation of the hydroxylase step in

10 the production of salicylic acid and to a feed-back step in which the salicylic acid increases the

11 production of H_2O_2 (Leon et al., 1995).

12   An elicitor, e.g., a bacterial or fungal pathogen, induces a cascade of reactions within a cell

13 (Figure 9-10b). Some of the lipid reactions are thought to be due to the opening of the Ca^{2+}

14 channels and the alkalination of the cell wall region. The oxidative burst due to H_2O_2 production

15 is believed to lead to the transformation of a small population of lipids into jasmonic acid, which

16 is a secondary messenger.

17   Hydrogen peroxide also has an oxidative role in lignification (Schopfer, 1994). In the

18 interaction of lignification and the beginning processes of hypersensitivity, pectinase produced

19 by the pathogen disrupts pectin and dissolves the cell wall. Fragments of the dissolved cell wall

20 trigger an increase in the transcription of peroxidases within the remaining cell wall, leading to

21 lignification, which is a cross-linking of the cell wall that does not use pectin. This prevents

22 further pathogen disruption of the wall and reduces its further entry into the plant cell.

23   It is believed that the first species generated through a one-electron reduction of molecular

24 oxygen is SO_2^-. That generation is carried out using a cytochrome b_6 by the NAD(P)H oxidase

25 located on the cell membrane (Auh and Murphy, 1995). In the acid region of the cell wall, SO_2^-

26 is converted by a protonation and dismutation to H_2O_2. The induced oxidative burst is believed

27 to play a role in stimulating the Cl^- and K^+ efflux, generating an alkalinization of the

28 extracellular space (Cazale et al., 1998). In the wall region, H_2O_2 is not especially toxic, as no

29 necrosis was reported in tobacco when 500 mM peroxide was infiltrated into the leaf tissue.

30 However, the production of salicylic acid and benzoic 2-hydroxylase can be induced with only

31 30 and 0.3 mM H_2O_2 respectively, indicating some metabolic signaling (Leon et al., 1995).

a.

Pathogen Recognition | **Defense Activation**

Free Radical Generation

+ | **Hypersensitive Cell Death**

H_2O_2 Generation

Accumulation of Benzoic Acid

+ Benzoic Acid
2-Hydroxylase Activation | **Local Resistance**

Salicylic Acid Accumulation

Systemic Salicylic Acid Increase | **Global Resistance**

b.

Ascorbate Peroxides (−)

Pathogen Kill

Cell Wall Structural Changes

Elicitor   Alkalinization OH^- Oxidative Burst

Cell Membrane

G Proteins (Rac2/Rap1A) → Open Ca^{2+} Channels → Phosphorylation Cascade

H^+ PM NADPH Oxidase   Lipid Hydroperoxides

Jasmonic Acid

Influx → High Ca^{2+} Levels → Transcription → Defense Genes

Signal Transduction

Figure 9-10.   Pathogen-Induced Hypersensivity. (a) The reactions leading to hypersensitive cell death and the formation of a global response of salicylic acid. (b) The cascade of the elicitor-induced reactions within the cell.

1   On the other hand, 1M H_2O_2 infiltrated into soybean will generate lipid peroxidation after 1 h

2   with a peroxidation rate of 15 nmol/g-FW h (Degousee et al., 1994). Cells react to the system[5]

---

[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.

1   and generate peroxide scavenging compounds within 1.5 to 2 hours, which appear to "mop up"

2   the excess H_2O_2 (Baker et al., 1995).

3   After O_3 exposure in birch, H_2O_2 has been found in the wall (Pellinen et al., 1999).

4   By using $CeCl_2$ as a cellular stain for H_2O_2 (as a cerium perhydroxide precipitate), Liu et al.

5   (1995) observed a gradual development of stain after 8 h of O_3 exposure (at 150 ppb). After 2 h

6   exposure, H_2O_2 stain was visible on the surfaces of both sets of mesophyll cells. Accumulation

7   of H_2O_2 stain continued for 16 h after exposure, suggesting a triggered-reaction rather than O_3

8   decomposition itself. H_2O_2 stain was present in the mitochondria, peroxisomes, and cytoplasm,

9   but not in the chloroplast. If methyl viologen (MV) was given to the leaves and then the leaves

10   were exposed to light, H_2O_2 stain could be observed within the chloroplast. This indicated that

11   the stain worked within the chloroplast if H_2O_2 were generated by the Mehler reaction

12   ($MV+O_2^-$). Thus, apparently, for birch, O_3 exposure does not generate excess H_2O_2 within the

13   chloroplast. Furthermore, these sets of experiments indicate that O_3 *per se* does not generate the

14   H_2O_2, but rather triggers stress-related H_2O_2 formation similar to what occurs in a pathogen

15   attack (the Reactive Oxidative Species or ROS reaction).

16   The presence of higher than normal levels of H_2O_2 within the apoplastic space is a potential

17   trigger for the normal, well-studied pathogen defense pathway. Figure 9-10b depicts such a

18   pathway and suggests that all the events and activation of pathways/genes caused by pathogen

19   defense could be observed when plants are fumigated with O_3. These events in Figure 9-10b will

20   be alluded to in later sections.

21   H_2O_2 has been linked to the hormone ABA-induced closure of the stomata by activating the

22   calcium influx in guard cells (Pell et al., 2000). The addition of H_2O_2 at a level of only 5 mM to

23   a guard cell preparation will cause a dramatic increase (ca. 9×) in electrical current at the

24   hyperpolarizing potential of –200 mV. Amounts as low 50 mM H_2O_2 will cause a less but still

25   sizable increase. Membrane stability is unaffected by the H_2O_2 and the activation of the channel

26   requires only about 2 to 3 minutes. Pell et al. (2000) also found that ABA induced the

27   production of H_2O_2 through ROS accumulation (also see Zhang et al., 2001).

28   Certain levels of ABA within the leaf lead to stomatal closure. The inactivation of a

29   phospho-tyrosine-specific protein phosphatase (ABI2) is an inhibitor of stomatal opening

30   induced by ABA but that enzyme is inhibited by H_2O_2 (Meinhard et al., 2002). This means that

31   H_2O_2 shifts the sensitivity of the stomatal opening to ABA (Figure 9-11), making the stomatal
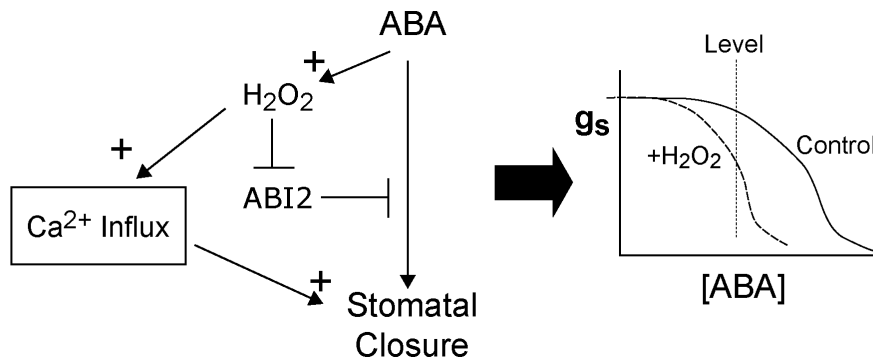
Figure 9-11. **The interaction of H_2O_2 and Ca^{2+} movements with ABA-induced stomatal closure. It is well known that certain levels of ABA within the leaf lead to closure of the stomata within the leaf. That level, however, can be shifted to make closure more or less sensitive to a given level of 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.**

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

1   complex more sensitive to ABA. Thus, for a given level of ABA present in the guard cell

2   complex due to environmental factors (e.g., low humidity, high air temperature, or low soil water

3   potential), the generation of H_2O_2 would (by inhibiting ABI2) induce a closure of the stomata by

4   increasing the sensitivity of the guard cells to ABA. In the past, it has been difficult to

5   understand why O_3 would often decrease conductance in some cases, but not always (Heath,

6   1994b). This interaction between H_2O_2 and ABA could help understand this complexity.

7

8   *Ethylene Reactivity*

9   Ethylene (ET) is produced when plants are subjected to biotic stressors e.g., attacks by

10   insects, fungi, and bacteria or abiotic stressors such as wounding or environmental stressors such

11   as heat, cold, or oxidative stress and O_3. If an O_3 stress has induced a wounding response with

12   ET release, then ET within the substomatal cavity could react with O_3, generating some

13   relatively noxious chemicals (see Figure 9-6). The relationship between O_3 injury and wounding

1 is supported by the observation by Mehlhorn et al. (1991) that an inhibitor of ET formation,

2 AVG (an inhibitor of ACC synthase, a committed step to ET production), would block ET

3 formation and inhibit visible injury. Other studies with polyamine (which is closely linked to

4 ET production), including those of Ormrod and Beckerson (1986) who fed polyamines to the

5 transpirational stream and prevented visible injury, suggested a close involvement of both

6 pathways to the production of visible injury. Both the lack of ET production or an increased

7 level of polyamines slowed or prevented visible injury.

8 This concept was taken another step by Langerbartles (Langebartels et al., 1991). The

9 linkage to the pathogen wound responses and visible injury is well established (Sandermann,

10 1996). Sandermann (1998) used a system of Bell B and W3 tobacco, plants with differential O_3

11 sensitivities, in which the O_3 exposure level was chosen such that the sensitive cultivar was

12 injured, while the tolerant one was not. This led to a marvelous control which could be used to

13 their advantage. They followed a time sequence to show that the rise of varied systems followed

14 the same order as for a pathogen attack (Heath, 1994a).

15 More recent studies, however, indicate that O_3 responses resemble components of the

16 hypersensitive response (HR) observed in incompatible plant-pathogen interactions (Sanderman

17 et al., 1998). The similarity to the HR response may be related to the occurrence of ROS in the

18 apoplast. The O_3-derived ROS apparently trigger an oxidative burst in the affected cells by an as

19 yet unknown mechanism. An oxidative burst is similar to one of the earliest responses of plants

20 to microbial pathogens and is an integral component in HR-related cell death (Overmyer et al.,

21 2000).

22 In plants exposed to O_3, ET synthesis is a result of the specific ET induction of the genes

23 encoding 1-aminocyclopropane-1-carboxylase synthase (ACS), one of the fastest and most

24 obvious responses to O_3, which has been mechanistically linked to the regulation of O_3 lesion

25 formation. Biosynthesis of ET inhibited with ACS inhibitors significantly reduced the induction

26 of lesion formation in plant leaves exposed to O_3 concentration (Vahala et al., 2003; Melhorn

27 and Welburn, 1987; Mehlhorn et al., 1991). Ethylene biosynthesis correlates best with O_3

28 (Vahala et al., 2003; Overmyer, 2000). These data support the concept that elimination of ET

29 formation will prevent visible injury.

30

1   *Ethylene-Interaction with Injury and Conductance*

2   Increased ET production by plants exposed to O_3 stress was identified as a consistent

3   marker for O_3 exposure decades ago (Tingey et al., 1976). They exposed more than 20 plant

4   species and cultivars to O_3 to determine whether the production of O_3-induced stress-ET could be

5   used to determine differences in plant sensitivity to O_3. Their studies suggested that increased

6   production of stress-ET correlated well with the degree of foliar injury that developed within

7   hours or days after O_3 exposure. The amount of ET released was exponentially related to the O_3

8   exposure. Furthermore, the amount of O_3-induced ET declined with repeated exposure,

9   indicating an acclimatization to O_3. This acclimatization effect associated with repeated

10   wounding has not yet been well described. The release of wound-induced ET is not linear with

11   time, but declines after the initial response (Stan et al., 1981), as is also seen after O_3 exposure

12   (Stan and Schicker, 1982). The stress-induced ET production correlates better with O_3 exposure

13   level than with exposure duration. In other words, peaks of high O_3 (rather than accumulated

14   dose) generate a higher rate of ET release, at least for a single O_3 exposure under an acute dose.

15   The production of ET after an O_3 exposure is thought to be a typical wounding response

16   (Tingey et al., 1975). Prevention of ET release may prevent the formation of visible injury

17   (Mehlhorn and Wellburn, 1987). However, the question arises as to whether this effect was

18   limited to the prevention of visible injury or if the chemicals used to prevent ET release closed

19   the stomata. Using *Glycine max* L., Taylor et al. (1988b) showed clearly that AVG did not

20   necessarily close stomata nor inhibit carbon assimilation *per se*.

21   The correlation of ET release with O_3-induced visible injury was likewise shown in pea

22   cultivars (Dijak and Ormrod, 1982). With O_3 exposure (generally 6 h at 0.3 ppm), the stomata

23   closed by ~50% within 3 hours after a dose of 3×10^{-5} mol cm^{-2} (with an average rate of

24   2×10^{-9} moles cm^{-2} s^{-1}, as calculated from their data). Both sensitive and insensitive cultivars

25   had a visible-linked-injury ET release, but sensitive cultivars scored higher both in visible injury

26   and in ET release after a given exposure.

27   Gunderson and Taylor (1988, 1991) used exogenous ET to alter the gas exchange of

28   *Glycine max* and found an exponential, but not simultaneous, decline of both stomatal

29   conductance and carbon assimilation with ET. Interestingly, the exogenous ET caused a slight

30   rise in difference of CO_2 within and without the leaf, indicating a lowering of internal CO_2,

31   which was not observed in the experiments of Farage et al. (1991) for O_3 exposure. Ethylene

1   does inhibit both stomatal conductance and carbon assimilation to some extent (Taylor et al.,

2   1988b). Thus, one could postulate that O_3 generates a wounding response with a production of

3   ET, which would, in turn, generate the change in stomatal conductance and photosynthesis.

4   Clearly, these multiple events may have confounded some earlier studies.

5

6   **9.3.3.4   Antioxidants Within the Apoplastic Space**

7   The first line of defense against O_3 is a closure of the stomata to exclude its uptake. This is

8   counter-productive for efficient photosynthesis, but some amount of closure limits the rate of O_3

9   deposition into the leaf tissue to allow for a secondary line of defense to detoxify the O_3. The

10 secondary line of defense involves a range of antioxidants, which are highly reactive to the types

11 of chemicals that can be generated by O_3. Several antioxidant proteins are stimulated by O_3

12 in *Plumbagini folia*, including glutathione peroxidase (GSH-P$_x$), SOD, and catalase. The

13 timescales for changes in their levels vary: some rise rapidly, while others rise more slowly.

14 The pattern of changes in these particular proteins varies greatly among different species and

15 conditions.

16

17 *Ascorbate Within the Cell Wall*

18   Most of the recent reports indicate that ascorbate within the cell wall is the real first line of

19 all defense. Ascorbate within the wall declines when the tissue is exposed to O_3 (Luwe et al.,

20 1993; Moldau, 1998; Turcsányi et al., 2000; Zheng et al., 2000). This decline appears to be

21 closely linked to the amount of O_3 penetrating the leaf tissue.

22   It has long been suspected that intracellular antioxidants play a role in preventing O_3-

23 induced injury to plant cells. Variation in the types of biochemical compounds present in the

24 apoplastic space can give rise to a multiplicity of reactions with O_3, but the predominant

25 biochemical species is ascorbate. Ascorbate is water-soluble, present in the solution where O_3

26 can dissolve, and is highly reactive. Unfortunately, a variety of antioxidants are found

27 throughout the cell and any measurements of one particular type within the total leaf tissue can

28 give misleading results. For example, ascorbate is present within the cell wall, within the

29 cytoplasm (Moldau, 1998; Burkey, 1999), and within the chloroplasts (Law et al., 1999); and

30 ascorbate can move between the cytoplasm and the cell wall with relative ease (Figure 9-12;

31 Bichelle et al, 2000). The total of all ascorbate pools are measured when the tissue is ground and
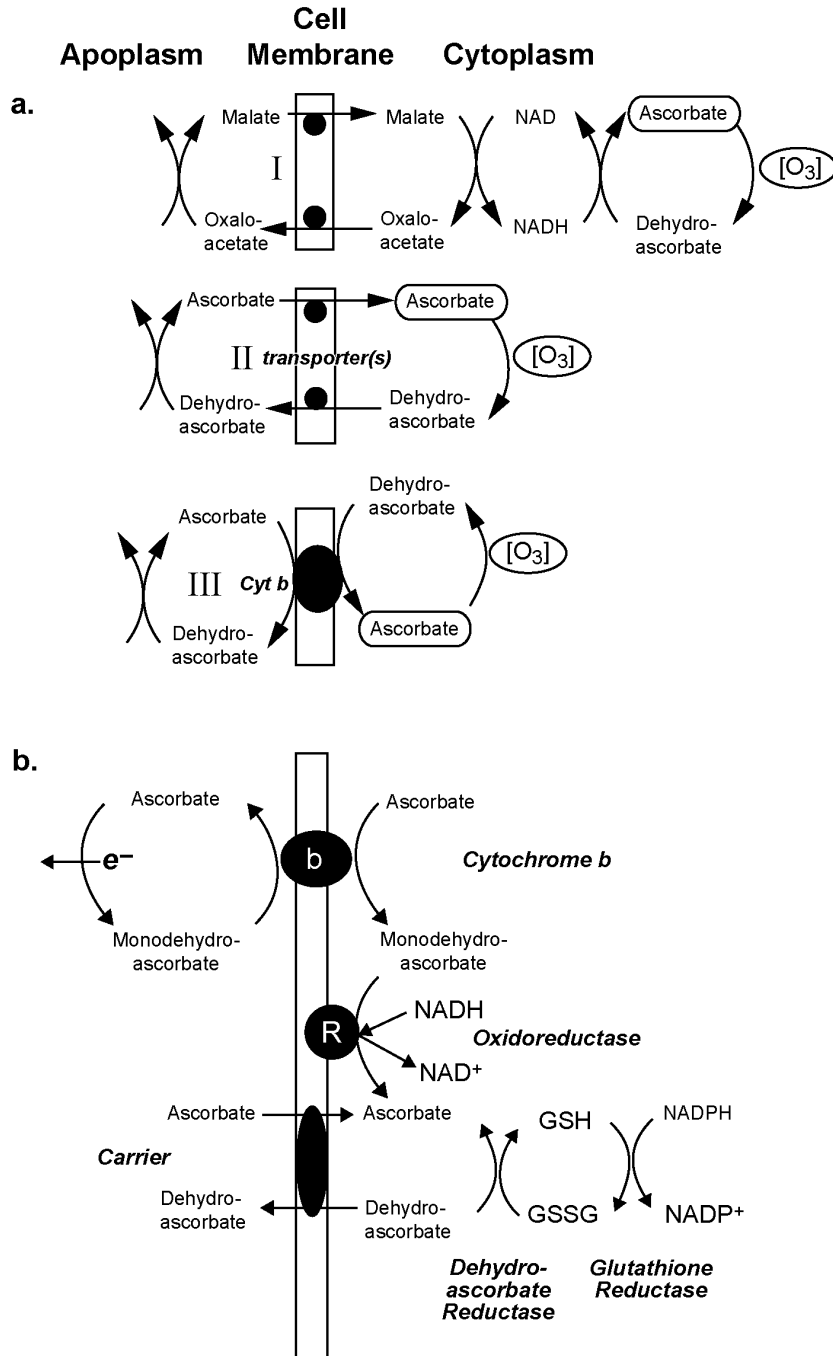
Figure 9-12. The reaction of ascorbate within the apoplasm of the cell wall and its ultimate reduction/oxidations.

    (a) Movements of reducing power (from Dietz, 1997).

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

1 assayed. If the cell wall ascorbate drops by 50% due to O_3 exposure but all other tissue

2 concentrations remain the same, the measurement of the total loss is dependent upon the amount

3 of ascorbate within the cell wall. Turcsányi et al. (2000) showed that, compared to the

4 concentration of apoplastic ascorbate, the rest of the cells contained about 38 times as much.

5 So a 50% loss of apoplastic ascorbate would be converted into only 2 to 3% loss of the total

6 ascorbate.

7   The ascorbate deficient *Arabidopsis thaliana* mutant has proven to be a powerful tool in

8 furthering the understanding of ascorbate biosynthesis in plants (Smirnoff et al., 2001). Three

9 classes of mutants were formed when *A. thaliana* seed was mutagenized with ethyl

10 methanesulfonate: (a) those deficient in SOD, (b) those that failed to accumulate more

11 antioxidant proteins upon increased O_3 exposure, and (c) those that were deficient (but not

12 depleted) in ascorbate. The low-ascorbate mutant type had 50 to 60% less ascorbate than the

13 wild type and displayed more foliar injury. This mutant is involved with the coding of the

14 GDP-D-Mannose pyrophosphorylase enzyme[6] in the Smirnoff-Wheeler pathway for ascorbate

15 biosynthesis. Smirnoff et al. (2001) also suggested that other pathways can produce ascorbate

16 without relaying upon the pyrophosphorylase step, but most probably at a slower through-put

17 rate, because any fully ascorbate-deficient mutant would be lethal, perhaps because of ascorbate

18 use as a cofactor rather than its antioxidant properties.

19   The ascorbate peroxidase (APX, which uses ascorbate to detoxify peroxides) family

20 consists of at least five different isoforms, with isozymes in the apoplastic and cytosolic space.

21 Furthermore, most forms of ascorbate can move through the plasma membrane (Figure 9-12;

22 Bichele et al., 2000), making the levels of all forms of ascorbate interdependent and able to at

23 least partially influence each other. Dehydroascorbate (DHA) can be broken down into other

24 smaller fragments easily in vivo and represents a continuous loss of ascorbate from varied parts

25 of the cell if ascorbate is allowed to remain in the oxidized form in some regions. In fact, the

26 turnover rate in leaves is estimated to be from 2 to 13% / hour, depending upon species and

27 developmental age (Smirnoff et al., 2001). There are apparently three pathways for ascorbate

---

[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.

1 turnover (Figure 9-12a). The typical reaction is a reduction of DHA into ascorbate from which

2 an oxidative step generates DHA. Pathway I requires a reductive step using NADH external to

3 the plasma membrane generated from internal malate using a malate/oxaloacetate transporter.

4 Pathway II uses a direct transporter of ascrobate/DHA. Pathway III moves the electron(s)

5 required through a cytochrome b system, maintaining two separate pools of ascorbate/DHA

6 within the cytoplasm and within the wall. Each of the pathways (Dietz, 1997) represented by

7 Roman Numerals in the Figure 9-12, require only one NAD(P)H molecule to reduce the DHA

8 molecule back to ascorbate. However, the transport properties and redox potential of the cell

9 differ for each pathway. The efficiency of the reduction of DHA is dependent upon the redox

10 coupling and the region in which the chemical species is located.

11   Turcsányi et al. (2000) exposed broad bean (*Vicia faba*) grown under two regimes in

12 duplicate controlled chambers: charcoal/Purafil filtered air (CFA) or (CFA) plus 0.075 ppm O_3

13 for 7 h/day for 28 days (chronic exposure) or exposed to 0.150 ppm for 8 h (acute exposure).

14 Responses of the two set of plants were similar except for stomatal conductance, which was 50%

15 lower in the chronic exposure plants. Plants grown under acute exposures developed visible

16 injury, while plants grown under chronic conditions developed no visible injury. Within an hour

17 of the start of the acute exposure, the stomatal conductance was reduced by nearly 40% and

18 assimilation was reduced by nearly 18% in the clean air plants; a reduction in conductance was

19 only 21% and assimilation 16% in the plants subjected to chronic O_3 exposures. The

20 assimilation was affected similarly in both cases, while the conductance showed less of a

21 percentage drop in the chronic O_3-exposed plants, beginning at a lower O_3 level. The similarity

22 of the assimilation indicated that the stomata were not limiting assimilation in either case before

23 acute exposure. More to the point, the decline in ascorbate in the apoplastic space due to the O_3

24 exposure was "…more often than not, on the borderlines of statistical significance." However, a

25 30% decline in ascorbate after 4 hours of acute O_3 in both cases was observed. This lack of

26 significance may be due to a relatively large standard error of the data, which in turn may be due

27 to the difficulty of extracting and measuring ascorbate from the apoplastic space in quantitative

28 terms.

1   The chemical reaction[7] of ascorbate and O_3 is given by the molecular rate constant of

2    $4.8 \times 10^7 \, M^{-1} \, s^{-1}$. This is some 50,000× the rate constant for ET. Of course, it depends upon the

3   relative concentration of ascorbate and ET, but it is likely that ascorbate is in higher

4   concentration than ET. One would then expect that the rate reaction of ascorbate with O_3 would

5   greatly dominate any possible reaction of O_3 with ET. For a concentration of ascorbate in the

6   range of 1 mM and for an O_3 concentration of about 0.1 ppm or 4.2×10^{-9} M, the detoxification

7   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)

8   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

9   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

10   1.3×10^{-5} M s^{-1} flux, which is less than 10% the detoxification rate.

11

12   ***Glutathione***

13   Many of the initial studies of O_3 exposure used high concentrations and measured only the

14   total sulfhydryl contents of the tissues. For example, in some of the earlier work, exposures of

15   tobacco to 1 ppm O_3 for 30 min induced a 15% loss of the total sulfhydryls (0.74 µmole/g-FW;

16   Tomlinson and Rich, 1968). These results are similar to other studies at high O_3 levels (Dugger

17   and Ting, 1970). It is now suspected that severe injury in their studies resulted in a massive

18   collapse of the cells releasing most of their internal constituents. Much of the oxidation thus

19   observed may have been the result of chemical oxidations of the O_3 that subsequently entered the

20   damaged tissue. Even under milder conditions, changes in sulfhydryl components have still

21   been noted and any sulfhydryl on the surface of the cell would be at risk due to its high reactivity

22   with O_3 (Mudd et al., 1969; 1997a). For example, the level of sulfhydryl compounds within the

23   protein of isolated chloroplasts declined about 66% when the chloroplasts were subjected to O_3

24   (about 1 µmole O_3) exposure (Mudd et al., 1971).

25   At this stage, it is important to note that there are inherent problems with metabolic studies

26   of full tissues. The first is that most organs have several different types of tissues. For example,

27   leaves have, at the minimum, epidermal and vascular tissues and two types of mesophyll cells.

28   Each type of cell may be metabolizing quite differently, producing very different levels of

---

[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.

1   metabolites and enzymes. Furthermore, most pathways are well regulated and after any small

2   disruption, the pathway tends to return to near its former stability. Changes in the level of

3   enzymes are likewise difficult to measure. Many enzymes function below their maximum

4   activities. Their speeds of reactions are often increased through regulation, rather than through

5   the production of more enzyme.

6   Glutathione is a three-amino acid peptide, which has antioxidant properties due to its free

7   reducing sulfhydryl group (G-SH). Glutathione is generally kept in its reduced form by

8   glutathione reductase (GR) with the reaction:

9

$$GS - SG(\text{oxidized}) + 2e^- + 2H^+ \rightarrow 2GSH \qquad (9\text{-}2)$$
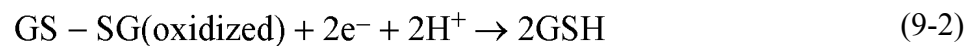
10

11   GR has six isoforms[8] within the chloroplast and six isoforms outside. The optimum activity

12   occurs at pH 7.8, suggesting it is located within the stroma of the chloroplast or the cytoplasm

13   rather than in the cell wall, which is at pH 4-5 (Madamanchi et al. (1992). Clearly, an increased

14   expression of GR (generated through transgenic implants) is important within the chloroplast to

15   prevent of some oxidations[9] (Aono et al., 1995; Foyer et al., 1995).

16

17   *Catalase*

18   Catalase, even though it breaks down H_2O_2, does not appear to protect plants from O_3

19   exposures. Two principle reasons may cause this lack of reactivity: (1) catalase has a high Km

20   for H_2O_2 and a low rate coefficient and (2) seems not to occur within the cell wall regions but

21   rather in the cytoplasm and peroxisomes (Buchanan et al., 2000). Only a few reports suggest

22   that catalase is increased by exposure to O_3 (Azevedo et al., 1998). Unfortunately H_2O_2 induced

23   by some forms of wounding in mesophyll cells can lead to induction of an increase in GSH and

24   the transient production of catalase (Vanacker et al., 2000). In general, it seems that catalase is

---

[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.

1   not really involved primarily in the defense of the cell due to O_3 attack but rather may be a

2   secondary response. The reaction of catalase (Scandalios, 1993) is as follows:

3

4
$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad K = 1.7 \times 10^{-7} \, M^{-1} \, sec^{-1}$$
(9-3)
5

6
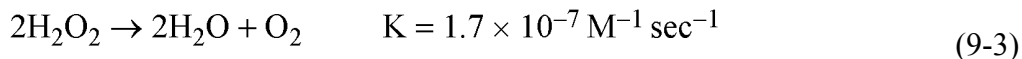
7   ***Superoxide Dismutase***

8   The varied compounds that O_3 can produce upon entering an aqueous solution are very

9   similar to those involved in the HR when plants are infected by an avirulent pathogen (Figure

10   9-10). The sequence of the plant response to the pathogen is: (1) recognition of the gene

11   products of the pathogen by the plant (elicitor), (2) generation of an immediate phytoresponse to

12   attempt to localize the attack and its products, and (3) generation of a systemic acquired

13   resistance (SAR) to subsequent attack by the pathogen. Inducible defense responses are

14   phytoalexin synthesis and production of pathogenesis-related proteins (PR). One aspect of this

15   total response is the production of O_2^- and H_2O_2 by the cell (Lamb and Dixon, 1997). The

16   elicitor can generate a transient alkalinization of the apoplast, up to pH 7.2, caused by a lowering

17   of the H^+-pump rate and a increase in the H^+-influx/K^+-efflux exchange. Other effects include:

18   a weak accumulation of transcripts for PAL (phenylalanine lyase); a larger and rapid induction

19   of glutathione S-transferase, GSH-P_x; oxidative cross linking of cell wall proteins which is

20   blocked by ascorbate acid; generation of localized apoptosis; and rapid influx of Ca^{2+}, which

21   activates apoptosis among other pathways (Lamb and Dixon, 1997). These effects seem to be

22   very similar to those induced by O_3 exposure (Sandermann, 1996; 1998).

23   The putative antioxidant enzyme SOD (Equation 9-4 and Table 9-8) catalyzes the

24   oxidoreductase reaction, which eliminates SO_2^- by dismutation (Bowler et al., 1992):

25

26
$$2O_2^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \qquad K = 2.4 \times 10^9 \, M^{-1} \, sec^{-1}.$$
(9-4)
27

28

29   The number, as well as the activity, of isozymes of each type of SOD in Table 9-3 can vary

30   with plant species. However, the isozymes that have been tabulated are Cu-Zn SODs, in cytosol

31   and chloroplast; Fe-SOD, active in chloroplast stroma, and Mn-SOD, active in mitochondrial

Table 9-8. 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

1. matrix (Karpinski et al., 1993). In the experiment demonstrating the activation of varied SODs,
2. there were three Cu-Zn SOD (*csd1*, *csd2*, *csd3*), three Fe-SODs (*fsd1*, *fsd2*, *fsd3*), and one
3. Mn-SOD (*msd1*) (Kliebenstein et al., 1998). Ozone sensitivity was determined by exposure of
4. 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
5. increases and most pronounced responses for mRNA and protein. Also, some increase in *csd3*
6. (thought to be peroxisomal) was induced when the plants were exposed to a high-intensity light
7. pulse; *msd1* was unresponsive to the environmental stressors used here, including O_3; and *csd2*
8. (thought to be chloroplast) showed little increase. The *fsd1* isozyme (present in the apoplasm)
9. showed a slight decrease. On the other hand, an early report on snap beans in which the
10. experimenters used EDU, N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N-phenylurea (Carnahan et al.,
11. 1978; Kostka-Rick and Manning, 1993) to prevent visible injury by O_3, 4 h O_3 exposure at
12. 0.45 ppm was correlated with an increase in general enzyme activity of SOD, i.e., the level rose
13. nearly 2.5× in 2 weeks at a level of 50 mg EDU per pot (Lee and Bennett, 1982). It is believed
14. that EDU may induce SOD, which then protects the plant. While gross assays of enzyme
15. activity have not proven to be very useful in understanding the mechanism of O_3 action, in a
16. well-crafted, long-term study involving ponderosa pine clones. Benes et al. (1995) stated that
17. "changes in antioxidant enzyme activity were not a consistent response to the O_3 fumigation, but
18. when observed, they occurred most often in the O_3-sensitive clone and in symptomatic,

1 fumigated branches…total (intra- and extracellular) activities of the antioxidant enzymes did not

2 appear to be good indicators of O_3 tolerance….”

3   Ozone exposure (70 ppb for 7 h/day for 14 to 42 days of exposure)[10] caused an increase in

4 POD and a decline in SOD with no change in APX. No GSH was detected, but the

5 concentration of (ASC + DHA) was at 20 to 25 nmol/g-FW of extracellular fluid, compared with

6 2.4 to 3.0 mmol/g-FW of cell fluid. Glutathione within the cell was only 100 to 170 nmol/g-FW

7 of cell fluid. While these results are what one might expect for POD, the decline in SOD and

8 lack of change in APX are not what would be expected if protection was provided by SOD and

9 ascorbate. Yet as noted, because the rate of SOD reaction is many times higher than the rate of

10 O_3 entry, there may be no pressure to increase the SOD level.

11   Some protection against visible injury (induced by 59 ppb daily mean O_3 for 14 h/day for

12 7 days) was observed in genetically modified tobacco plants with excess chloroplast SOD[11] (2 to

13 4 times higher), but less protection was observed in plants that had an excess of mitochondrial

14 SOD ($8\times$ higher) (Van Camp et al., 1994). In all lines, the conductance of the leaves dropped

15 about 50%, compared with the unmodified plants. There was a correlation with age of leaf (less

16 injury in younger leaves) that corresponded to that found in spruce trees in which the amount of

17 SOD declined in relation to the longer that needles were held on the tree (Polle et al., 1989).

18 A slightly different study, however, found no O_3 protection with varied SOD within the needles

19 (Polle and Rennenberg, 1991). Interestingly, in maize, the synthesis of SOD (any form) was not

20 stimulated by O_3 exposure (at 0.50, or 0.75 ppm for 8 h, variable times thereafter) but was by

21 exposure to 90% O_3 (Matters and Scandalios, 1987). It may be that this high level of O_3 does not

22 affect the SOD, or perhaps it stimulates and degrades the enzyme simultaneously.

23   The conclusions to be drawn from these results are not obvious. There seems to be SOD (a

24 Cu-Zn form) present in the apoplastic space of some plants, but it does not necessarily rise with

25 O_3 exposure. Thus, either its concentration is sufficient to provide protection or it is not needed.

26 Over-expression of any SOD in other organelles may play a role, especially in the chloroplast

---

[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/m^2 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 500,00 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.

1   (Cu-Zn or Fe forms); but it may be playing a secondary role due to other effects of O_3 that

2   generate conditions in which light can overload the chloroplast and generate detrimental

3   circumstances, including the production of SO_2^-. In addition, SOD is developmentally expressed

4   in varied concentrations, so that long term exposure to O_3 may alter each leaf's developmental

5   age and, in turn, alter what level of SOD is observed. In any case, SOD does not seem to be the

6   primary antioxidant system to protect against O_3.

7

8   ***Changes to the Plasmalemma***

9   Reports of "peroxidation" generally occur within unicellular organisms subjected to very

10 high levels of O_3 (in *Chlorella* [Frederick and Heath, 1970)] and in *Euglena* [Chevrier et al.,

11 1990)]). Heath (1987) determined that by the time biochemical events were altered and MDA

12 was produced in *Chlorella*, little permeability remained in the cells and most metabolic

13 pathways were greatly disrupted by the subsequent loss of substrates. In fact, MDA production

14 was concurrent with a high O_3 uptake by the cell, indicating a complete opening of the cell and

15 associated with the concurrent inability to plate the cells on a glucose median (indicative of cell

16 death). Heath reached the conclusion that no one had proven that lipid oxidation was in any way

17 a part of the initial reactions of O_3 with the cell, a conclusion confirmed by Mudd et al. (1997a).

18 An excellent review regarding the initial action induced by O_3 within a plant (Kangasjärvi et al.,

19 1994) should be consulted. There is little data to show that lipids are attacked by O_3 in any

20 living system that was not previously severely injured by O_3. Most of the data suggesting lipid

21 attack by O_3 has been demonstrated in plants subjected to O_3 concentrations of 0.5 to 1.0 ppm for

22 several hours, during which gross wilting of the plant tissues usually occurs, suggesting extreme

23 water loss. It is not surprising that lipid and protein injury is observed under these conditions.

24 While those reports were useful in the 1960s and 1970s, they are not especially insightful now

25 when ambient levels of O_3 are rarely above 0.2 ppm.

26

27 ## 9.3.4 Wounding and Pathogen Attack

28   The decline of an enzyme is more difficult to measure than the rise of a new enzyme; an

29 increase from 0 to 2% may be within the precision of any assay, but a decrease from 100% to

30 98% is often masked by simple variation of the assay. Thus, measuring enzymes, which are in

31 great abundance in pre-fumigated tissue, is a risky operation. On the other hand, if O_3 induces a

1   general physiological change that has characteristics similar to other well-studied, stress-induced

2   changes, then O_3 studies could "piggy-back" onto those studies to gain insight into the full scope

3   of metabolic alterations. It is now becoming clear that wounding and pathogen attack of plants

4   are similar to O_3-induced changes in plants, and a reasonable hypothesis is that O_3 must induce

5   one or more of the first steps seen in the wounding/pathogen-attack response.

6   Systemic acquired resistance (SAR) has been heavily investigated, and DNA probes have

7   existed for some time for a series of expressed genes (see Table 9-9). Several enzyme classes

8   are associated with O_3 injury, including glucanases and peroxidases and others, such as the PR

9   proteins and chitinases. Thus, strong evidence exists from enzyme function and genetic material

10   that O_3 induces an activation of a SAR-like response.

11   Mehdy (1994) described a model of how an elicitor produced by the pathogen attack

12   activates a G-protein, which opens the inward-flowing Ca^{2+} channel. The flow of Ca^{2+} into the

13   cytoplasm raises the internal level (at the µM level) and activates a protein kinase that increases

14   the activity of the plasma membrane NAD(P)H oxidase and generates O_2^-. Superoxide

15   dismutase converts O_2^- into H_2O_2. Both O_2^- and H_2O_2 are responsible for the active oxygen

16   species response, which is believed to be a defense mechanism to kill the pathogen. In this

17   normal defensive reaction, a subsequent system induces either localized lipid peroxidation per se

18   or a membrane lipase to produce jasmonic acid or inositol triphosphate, which act as secondary

19   messages to activate the defense gene products.

20   Booker et al. (2004) found that G-proteins might be involved in the perception of O_3 in the

21   extracellular region using *A. thaliana* G-protein null mutants. The activation of a passive inward

22   flow of Ca^{2+}, e.g., by an O_3-induced response, would serve the same function as activation of the

23   G-protein. Once the level of cytoplasmic Ca^{2+} rises, all else follows. It is suspected that

24   exposure of plants to O_3 does just that, as Castillo and Heath (1990) demonstrated — the in vivo

25   fumigation of bean plants both inhibits the outward-directed ATP-requiring Ca^{2+} pump and

26   increases the passive permeability of Ca^{2+}. It was thought that the calcium transporter system

27   has a sensitive sulfhydryl group which, if oxidized, would alter normal Ca^{2+} movements. In

28   addition, Dominy and Heath (1985) observed that the K^+-activated ATPase (believed to be

29   involved in K^+ transport) was inactivated by in vivo exposure to O_3 and that inactivation was

30   traced to a sensitivity sulfhydryl. Mudd et al. (1996) argued that several amino acids are very

31   sensitive to O_3, including any with an exposed sulfhydryl. Thus, the O_3-induced change in Ca^{2+}

Table 9-9. 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. (1988b)
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. (1990a)
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. (1988a)
PR-1 basic	Basic isoform of acidic PR-1	Payne et al. (1989)
Basic class III chitinase	Homologous to cucumber chitinase (Metraux et al., 1989), structurally unrelated to PR-3	Lawton et al. (manuscript in preparation)
Acidic class III chitinase	Extracellular; approximately 60% identical to basic isoform	Lawton et al. (manuscript in preparation)
PR-O'	Acidic, extracellular b-1,3-glucanase; approximately 55% identical to PR-2 group	Payne et al. (1990b)
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)
SAR 8.2	Unknown function; cloned by ± screen of cDNA library from secondary leaves of TMV-infected plants	Alexander et al. (manuscript in preparation)
Acidic peroxidase	Extracellular; lignin-forming	Lagrimini and Rothstein (1987)

1   permeability may be the trigger to most, if not all, the wounding responses. However, the

2   difficult problem of proving that the cytoplasmic Ca^{2+} change is the first event in O_3 injury

3   remains.

4   　　　Some wound- and pathogen-induced genes that are activated or repressed in *Arabidopsis*

5   *thalania* are found with DNA arrays (Cheong et al., 2002). While these responses may not be

6   uniform for all plants, they suggest the possibility of wide-ranging gene changes that may occur

7   with a simple wound and that those changes are wide-ranging and diverse. As an example, these

8   responses are related to hormonal responses that are related to jasmonic acid, ET, and auxin

1   pathways; signal transduction responses; and transcription factors for a variety of pathways.

2   The involvement of ET in wounding and pathogen attacks is discussed in Section 9.4.3.2.

3

4   **9.3.4.1 Peroxidases**

5   Increases in cytosolic and apoplastic peroxidase activity in response to O_3 are often

6   observed, but the reasons and outcomes of these changes have yet to be fully explained.

7   Increased activity is frequently correlated with O_3 injury. Dass and Weaver (1972) observed that

8   increases in peroxidase after O_3 injury was similar to that observed for plant infection by a virus.

9   Tingey et al. (1975) observed a 35% decrease in peroxidase activity immediately following O_3

10   exposure; however, within 24 to 48 h, activity had increased significantly and was above control

11   level and remained there throughout the remainder of the study. Dijak and Ormrod (1982) also

12   observed increases in peroxidase activity when two O_3-sensitive and two O_3-resistant varieties of

13   garden peas (*Pisum sativum*) were exposed to O_3. Peroxidase activity was not related to cultivar

14   sensitivity nor to visible injury. Unfortunately, there are many peroxidases (Birecka et al., 1976)

15   therefore, any general increase is not specific. In ET-treated leaves, peroxidase reaction

16   products were found between the plasma membrane and the cell wall, suggesting that ET itself

17   could induce peroxidase activity (Abeles et al., 1989a,b; Birecka et al., 1976).

18   At the same time, others examined peroxidase reactions in general and found two types of

19   peroxidases (designated as acidic or anionic and basic or cationic, EC 1.11.1.7, but also listed as

20   EC 1.14.18.1). Many types of peroxidases are located in diverse organelles, and each seems to

21   be activated by different conditions (e.g., pH for anionic and cation types and substrates such as

22   guaiacol, syringaldazine, and ascorbate). Peroxidases belong to at least two groups, which

23   catalyze two separate reactions: (1) the reaction of H_2O_2 with ascorbate to form DHA, discussed

24   earlier (Thom and Maretzki, 1985), which is regenerated by plasma membrane electron transport

25   using a dehydrogenase (Gross and Janse, 1977) that is now believed to be a malate/oxaloacetate

26   shuttle through the membrane coupled to a NAD(P)H-cytochrome-b-reductoxidase; and

27   (2) the reaction with coniferyl alcohol (from phenylalanine through phenylalanine ammonia

28   lyase) to form lignin within the wall. The anionic peroxidase thought to be involved with

29   lignification is within the cell wall (Buchanan et al., 2000; Taiz and Zeiger, 2002). Some basic

30   peroxidases are maintained within the cell, while some are external to the cell. After wounding

31   (Lagrimini and Rothstein, 1987; Gasper et al., 1985), some basic peroxidases can be activated by

1   processes leading to the synthesis of stress ET (Yang and Hoffman, 1984) and/or by excess Ca^{2+}

2   (Gasper et al., 1985). Elicitor treatment of plants change a series of peroxidases, some of which

3   are similar to those seen in O_3-induced changes (see Table 9-9).

4   The formation of lignin is due to the phenylpropanoid metabolism (Buchanan et al., 2000).

5   Tyrosine and phenylalanine are converted to cinnamic and *p*-coumaric acid, which are in turn

6   converted to *p*-coumaryl, coniferyl, and sinapyl alcohols, and then into lignins. Hence, the

7   peroxidase activity is often measured by one of these substrates (Espelie et al., 1986; Gasper

8   et al., 1985). However, it is questionable whether apoplastic peroxidase activity is limiting for

9   lignification; laccases have a prominent role as well. Also, availability of monolignols is critical

10 for core lignin formation, and it is unclear whether levels of these metabolites change in

11 response to O_3. Studies by Booker (Booker et al., 1991, 1996; Booker and Miller, 1998; Booker,

12 2000) indicated that O_3 did not increase core lignin concentrations in foliage of loblolly pine,

13 soybean, or cotton; although levels of phenolic polymers and cell wall-bound phenolics were

14 elevated in soybean. Increased phenolic polymers appear to be lignin in acid-insoluble lignin

15 assays and may well be responsible, along with polyphenol oxidase, for the stippling injury

16 observed in O_3-treated plants. Cell wall function implies the transport of peroxidase molecules

17 out of the cell and, most likely, the regulation of their activities within the wall space. These

18 extracellular peroxidases may be observed by vacuum infiltration of buffer into leaf air spaces

19 and subsequent centrifugation of the tissues to remove the buffer with the apoplastic enzymes,

20 that wash out (Castillo and Greppin, 1986). However exposure to O_3 induces important changes

21 in the plant. For example, extracellular peroxidase activity in *Sedum album* leaves increased

22 nearly 3-fold over that in the control plants after 2-h exposure to 0.40 ppm (Castillo et al, 1984).

23 This O_3-induced increase of extracellular peroxidase appears to be under the control of Ca^{2+}

24 (Castillo et al., 1984; Heath and Castillo, 1987). Initially, no effect on the anionic activity as

25 measured with syringaldazine (specific electron donor for lignifying peroxidases) was observed,

26 yet 21 hours later, the anionic peroxidase activity was increased, whereas the cationic (ascorbate

27 measured) peroxidase activity was decreased in O_3-treated plants. This suggests an immediate

28 response (ascorbate peroxidase activation) and a secondary response that activates the lignifying

29 peroxidase via gene activation.

30   The rapid response of cationic peroxidase after O_3 exposure may not result from de novo

31 protein synthesis but from the secretion and direct activation by Ca^{2+} ions of enzyme molecules

1 already present in the tissue. Cationic peroxidases might attack the peroxides and, in this

2 manner, act as a detoxifying agent with ascorbate as the substrate in the apoplasm. The effect of

3 Ca^{2+} upon peroxidase activity is stronger at low H_2O_2 concentrations (Penel, 1986). Thus, one

4 can imagine that, when the H_2O_2 concentration is low, this peroxidase activation would have a

5 greater in vivo importance. Furthermore, the secretion of cationic peroxidases into the free

6 spaces as a result of O_3 treatment is accompanied by a simultaneous release of at least one of its

7 natural substrates (ascorbic acid); this cationic peroxidase exhibits a much higher affinity

8 towards ascorbate (up to 6-fold) than the anionic isozyme (Castillo and Greppin, 1986).

9

10 **9.3.4.2   Jasmonic Acid and Salicylic Acid**

11   Salicylic acid (SA) and jasmonic acid (JA) are considered to be regulators of the plant

12 defense response (Figure 9-13) (Buchanan et al., 2000). They tend to respond more slowly than

13 ET, causing widespread effects in the plant tissues. Both seem to be heavily involved in

14 responses of the plant to O_3, once again linking the pathogen/wounding defense to O_3-induced

15 injury; however, their roles are far from clear.

16   One of the lipoxidase isoforms is activated by pathogen infection (POTLX-3) within 6 h

17 and accumulates for a week (Kolomiets et al., 2000). This enzyme is the first stage of the JA

18 pathway which leads to 13-hydroperoxide linolenic acid (HPOT) which is converted either to

19 allene oxide through AOS or to C6 aldehydes through hydroperoxide lyase. These aldehydes act

20 as signaling agents via systemin (Sivasankar et al., 2000) or volatile odiferous compounds

21 (oxylipins) that have been implicated as antimicrobial toxins (Froehlich et al., 2001).

22 Interestingly, these compounds seem to target the chloroplast envelop where they interact with

23 its metabolism. As HPOT and AOs are both implicated in plant defense and are activated by O_3,

24 these interactions may be related to how chloroplast enzymes and their mRNAs are involved in

25 O_3-induced injury.

26

27 **9.3.4.3   Stress-Induced Alterations in Gene Expression**

28   Early studies addressed the qualitative and quantitative effects of O_3 on protein metabolism

29 (Harris and Bailey-Serres, 1994). Subsequent reports suggested that the physiologic and

30 metabolic consequences of exposure to O_3 was, in part, mediated by increased gene expression.

31 A summary of the gene-linked changes in proteins induced by SAR may be seen in Table 9-9.
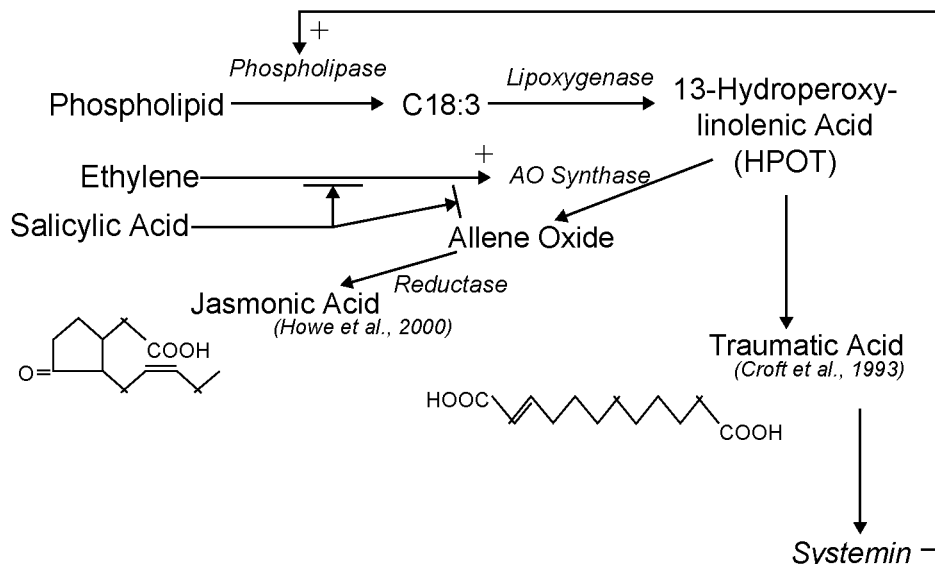
Figure 9-13. **The pathway leading from phospholipids to jasmonic and traumatic acid. The role of lipoxygenase and the production of a hydroperoxyl moiety from the unsaturated fatty acid is clearly demonstrated. More importantly, several of the enzymes within this pathway have been shown to be activated by oxidative conditions including O₃ exposure. The production of both of these acid species could lead to a general global response of a whole plant to the O₃ exposure of a single leaf.**

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

1   Of particular note are the productions of PR proteins, chitinase, glucanase, and acidic

2   peroxidases that appear to be common markers used in many O_3 studies. A summary of varied

3   proteins as measured by changes in the mRNA in *A. thalania* induced by O_3 exposure is shown

4   in Table 9-10. While studies on *Arabidopsis thaliana* required high concentrations of O_3 to

5   produce a response, the levels reported in most of the studies did not induce visible injury. The

6   types of messages induced included glutathione S-transferase, PAL, ACC synthase, SOD, and

7   some PRs. Slower increases in messages are seen for other PR and SAR-senescence proteins.

8   Declines in messages were observed for varied chloroplast enzymes, including those for Rubisco

9   and chlorophyll binding proteins. A few new proteins were found — a casein kinase and three

10   plasma membrane proteins. It is interesting to note that few messages for "new" proteins were

11   generated by O_3 exposure.

Table 9-10. 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		Sharma (1994)
300 ppb for 6 h daily	10 bands of 10 RNA					AtOZI1 >> casein kinase II	Sharma (1995)
200/500/1000 ppb for 2 h	Wilting (8 h); premature senescence					3 plasma membrane proteins: 75-, 45-, 35-kDa peptides	Tokarska-Schlattner (1997)
350 ppb for 1-6 h	Ethylene production; downward curvature; water logging	ACS-6			ACS-1, -2, -4, -5		Vahala (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 (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 (1999)
160 ppb for 3-72 h	Early senescence	GST1,VSP2;	MT1		CCH		Mira (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 (1995)

Table 9-10 (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 (2002)
250 ppb for 6 h	Lesion initiated on margin and spread inward					rcd1, on chromosome 1, single Mendelian trait	Overmyer (2000)

Abbreviations used in Tables 5 and 6.

GST = Glutathionine synthase
PAL = Phenylalanine ligase
PR-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 =
ERD1 = Ethylene response
SAG21 = Senescence
rbcS = Rubisco small subunit
MT1 = Mitochondria

1	 The working hypothesis is that O_3, which is not eliminated by antioxidants in the cell wall,

2	alters the properties of the plasma membrane. Specific polypeptides, indicative of these

3	antioxidants, are induced. If specific receptor molecules or channels on the membrane are

4	affected, the ionic balance within the cytoplasm is changed, leading to altered transcription or

5	translation of the genes controlling those and other types of polypeptides. Once this membrane

6	disruption occurs, the cell must mobilize repair systems to overcome the injury. Thus, carbon

7	and energy sources once destined for productivity, must be used in repair processes. Some of

8	these repairs are thought to result from the induction of specific genes. Photosynthesis is

9	inhibited by direct inhibition of some of the enzymes, through byproducts of O_3 attack or by

10	altered ionic balance. At the very least, the decrease in photosynthesis is a result of an

11	O_3-induced decrease in *rbcS* mRNA.

12

13	## 9.3.5 Primary Assimilation by Photosynthesis

14	### 9.3.5.1 Photooxidation: Light Reactions

15	 Photooxidation refers to the oxidation of chlorophyll within the light reaction due to an

16	imbalance between light absorption and the CO_2 use to produce carbohydrates. It was

17	discovered in the 1920s and studied under the concept of chlorophyll bleaching and photo

18	autooxidation (Asada, 1999; Rabinowitch, 1945). What generally occurs is that electron transfer

19	from H_2O to NADPH declines, and a light reaction overload occurs. The slowdown of electron

20	transfer may also be due to inhibition of the dark reactions, through the poor use of small

21	molecular weight carbohydrates or a lowered amount of the fundamental substrate CO_2.

22	To counteract these detrimental reactions, a series of "antioxidant" reactions exist, which

23	eliminate the buildup of oxidative intermediates.

24	 A lowered CO_2 level, which can be caused by stomatal closure (Heath, 1996), blocks the

25	use of reduced plastoquinone (PQH_2) in Photosystem II through NADP reduction in

26	Photosystem I (Hankamer et al., 1997). The buildup of PQH_2 reduces the amount of Q_A,

27	resulting in a buildup of $P_{680}^+|Pheo^-$ species (the primary photoact). The inability to reduce this

28	radical leads to injury to the D_1 protein (32 kDa) and its fragmentation into 23-, 16-, and 10-kDa

29	fragments (Hankamer et al., 1997). Ozone exposure of bean plants leads directly to the loss of

30	this D_1 protein (Pino et al., 1995). The loss of D_1 stimulates the production of new D_1 (and its

31	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 with oxidized products to produces 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. Farage et al. (1991) and Farage and Long (1999) studied these reactions in wheat and concluded that alterations to the dark reactions were much more common.

9.3.6 Alteration of Rubisco by Ozone: Dark Reactions

A large body of literature shows that O_3 exposure induces a decline in Rubisco (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). Because Rubisco plays such an important role in the production of carbohydrates (Figure 9-14), any loss may have severe consequences for the plant's productivity.

The study by Noormets et al. (2001) used an exposure system of plants in a FACE exposure facility in which areas of ambient (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. Two clones of aspen (O_3-tolerant and -sensitive) were used in all four cases. The study recognized that leaf age was important in the variation and tried to control for it by grouping the data by leaf plastochron index[12]. These data, taken over many days using the LiCOR 6400, confirmed earlier observations that O_3 has the greatest effect on older leaves after causing a decline in assimilation and in conductance. They also 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, 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). The level of Rubisco was not measured as frequently as the assimilation and conductance, but the significant (5%) increase in Rubisco was only observed in older leaves for both clones. More to the point was

---

[12]The plastochron index for the leaf measures the leaf age by degree of expansion rather than simply by chronological time.
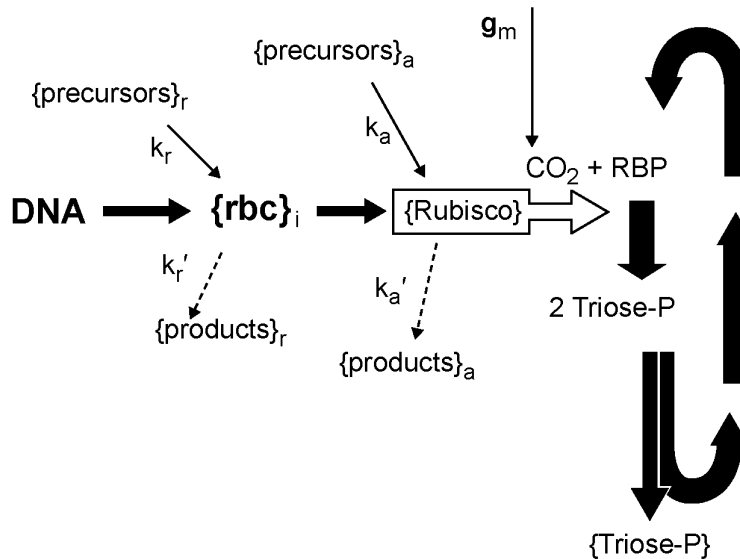
Figure 9-14. **The production of Rubisco and its Calvin Cycle pathway reactions. Two peptides are used to build Rubisco: *rbcS*, the small subunit produced by DNA within the nucleus; and *rbcL*, the large subunit produced by DNA within the chloroplast itself. Clearly both polypeptides must be closely regulated to produce the enzyme in a coherent manner. Furthermore, at least five isoforms of DNA can produce *rbcS*, each of which is regulated by a different promotor region.**

1   that the stomatal limitation[13] was not altered by O_3 exposure, with or without excess CO_2. It is

2   critical to point out that mesophyll conductance is directly linked to internal CO_2 level[14]. So if

3   C_i/C_o is constant and g_s declines, then g_m must likewise decline. If, as it is argued, Rubisco

4   levels are constant or at least increasing, then a regeneration of RuBP must be the cause of the

5   decline in g_m. Farquhar et al. (1980 were more concerned with high levels of CO_2 and had little

---

[13]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.

[14]Respiration is generally small at saturating A and often is ignored. By transforming $\{A = g_s (C_o - C_i)\}$ 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.

1   to say about O_3 exposure. One thing is certain however, the use of clones using different aged

2   leaves is a preferred method for this type of investigation.

3   The level of carbohydrate within the cell has an effect upon the amount of mRNA for

4   Rubisco (*rbcS*). Experiments by Krapp et al. (1993) indicated that a decline in carbohydraft

5   levels is probably due to the increased production of control metabolites, such as fructose

6   2,6-bisphosphate, which can shut down important sugar production pathways. This report also

7   leads to a measure of half-time for the decline in *rbcS* of about 2 days[15] when 50 mM glucose is

8   added to a cell suspension of *Chenopodium*. Also, the carbohydrate level was increased by cold

9   girdling the petioles of tobacco and potato using intact plants. The levels of carbohydrate nearly

10   doubled in 5 days and the level of *rbcS* declined rapidly (reaching 25% after 12 h). A decline in

11   Rubisco followed, but more slowly (with an estimated half-time of about 108 h after a lag of at

12   least 12 hours). This, of course, is expected; the level of the enzyme would decline slowly with

13   a lag after a loss of the message.

14   A better estimation of the half-life of *rbcS* can be found in the Jiang et al. (1993) study of

15   the destabilization of the message by an antisense message. The wild type *rbcS* in tobacco had a

16   half-life of about 5 h compared to that in the mutant with the antisense. It was argued that the

17   antisense message increased the degradation of the normal *rbcS*. The estimated half-life of *rbcS*

18   under O_3 fumigation is about 1 hour (Pell et al., 1994). Although comparisons of these diverse

19   systems can not be easily made, the normal half-life of *rbcS* may be closer to 5 to 10 h; and O_3

20   fumigation does not simply stop the transcription of DNA, but rather it alters the rate of

21   degradation, either independently of, or simultaneously with, transcription.

22   Williams et al. (1994) developed a correlation between the levels of ABA after water stress

23   in *Arabidosis thaliana* leaves and the loss of *rbcS*. Although their data were not quantitative, the

24   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

25   about 2 to 4 hours. Their work suggests that drought stress may alter the CO_2 metabolism by

26   changing enzyme relationships much more than by merely closing the stomata. If an ABA rise

27   is lowering *rbcS*, *rbcS* may not be a good marker of O_3 fumigation except under highly

28   controlled conditions.

29

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

9.3.7 Carbohydrate Transformations and Allocation

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 past 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.

Trade-offs 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

1   conditions retained their leaves longer and had a greater respiration rate within those leaves

2   (Maurer and Matyssek, 1997). Again, one must be careful in comparing short-term versus long-

3   term exposures. Grulke et al. (2001) observed that maximum concentrations of carbohydrates in

4   1-year old needles that had not abscissed due to early senescence declined when subjected to

5   year-long exposures along an increasing pollution gradient. Furthermore, the monosaccharide

6   concentrations in fine roots (along with starch) were largely decreased, suggesting that needle

7   sugars were limiting, leading to root sugar limitations. However, determination of the total

8   productivity and detailed balance of carbohydrate was impossible, because these were older,

9   larger trees and the data were taken over a full growing season. For a shorter-term exposure of

10   9 days, Smeulders et al. (1995) observed that O_3 appeared to increase the retention of labeled

11   photosynthates within the needle, and, at higher exposures (400 versus 200 or 0 mg/m^3), the total

12   starch within the needle decreased, suggesting that less carbohydrate was produced within the

13   cell or perhaps that it was in compounds not measured.

14   Studies with Pima cotton, aspen (*Populus* spp.) and bean seedlings (*Phaseolus vulgaris*)

15   indicate that acute O_3 exposures inhibit export of the current assimilate that provides

16   carbohydrates to the roots from source leaves of cotton as well as recent assimilate from the

17   older leaves of aspen and bean (Grantz and Yang, 2000). Grantz and Yuan (2000) attempted to

18   distinguish between potential mechanisms of O_3 phytotoxicity operating at the level of the whole

19   plant. Four hypothesis were tested by fumigating cotton: (1) O_3 exposure reduces leaf pools of

20   soluble sugars; (2) pruning leaf area and reducing source strength to match that of O_3-treated

21   plants reproduces O_3 effects; (3) pruning lower leaf area more closely reproduces O_3 effects than

22   pruning the upper leaf area; and (4) manipulating plant age and, thereby, plant size to match

23   O_3-treated plants reproduces O_3 effects. All were shown to be incorrect. Under each of the

24   above conditions, Grantz and Yang (2000) reduced the amount of foliage to match that caused

25   by O_3 injury. While the treatments reduced the biomass and leaf area, they did not alter biomass

26   allocation nor root function. They concluded that a simple loss of foliage does not induce the

27   changes in translocation to the roots to the same extent as does O_3 injury.

28   This finding by Grantz and Yang (2000) is important in that it suggests that O_3 can trigger

29   a plant-wide response that may be linked to alterations in signal transduction and the generation

30   of whole plant signals. Stitt (1996) suggested that "…allocation is regulated by long-distance

31   signals that act to influence growth of selected sinks and to modify the delivery of resources to

1   these sinks in parallel." Cooley and Manning (1987), citing Mc Laughlin and McConathy

2   (1983), suggested three possible ways that O_3 fumigation might alter translocation:

3   (1) malfunction of the phloem loading process, (2) increased allocation to leaf injury repair, and

4   (3) an altered balance between the leaf and sinks caused by reduced carbon fixation and a greater

5   demand for assimilate in the leaf.

6   Ethylene has been shown to reverse this sugar inhibition of development and to be

7   antagonistic to the ABA effect (Finkelstein and Gibson, 2002). However, these effects depend

8   greatly upon the developmental stage of the plant. Thus, the balance of the effectors (sugars,

9   ABA, and ET) may interact to generate the variation observed in the O_3-induced productivity

10   decline. For example, O_3 fumigation can induce a shift in the carbon transfer between roots and

11   shoot; and this shift can be amplified by mild drought (Gerant et al., 1996). Furthermore a

12   regulation of source-sink relations with the defense responses induced by elicitors was observed

13   by wounding the leaves of *Chenopodium rubrum*. Ethylene appears to be able to repress the

14   expression of extracellular invertase, which is critical for control and down-loading of sucrose

15   derived from the translocational stream (Roitsch, 1999; Lindow et al., 1996). In addition, the

16   development of *Arabidopsis* at high concentrations of glucose or sucrose is arrested by

17   increasing the ABA level (Coruzzi and Zhou, 2001).

18   Clearly more work is needed on the interactions between assimilation, translocation, and

19   source/sink relations with O_3 exposure. In these interactions, one must be aware of the

20   developmental age of the plants and their phytohormonal status.

21

22   **9.3.7.1   Lipid Synthesis**

23   Heath (1984) summarized several early reports of O_3-exposure induced lipid alterations.

24   Most concerned the production of MDA as a measure of lipid oxidation as well as the loss of

25   unsaturated fatty acids. However, a series of experiments by Sakaki and coworkers concentrated

26   on one type of fumigation system and one metabolic pathway. This literature provides the best,

27   most complete story with regard to lipid metabolism and O_3 fumigation and indicators that O_3

28   injures cellular membrane systems via lipid destruction.

29   Sakaki and coworkers used spinach, which is a sensitive plant but which has not been

30   much evaluated with respect to O_3 fumigation. While the O_3 level was high (0.5 ppm), enough

31   work has been done to be able to "tease apart" what is happening. The first paper showed that

1   chlorophyll bleaching does not begin until the plants have been exposed to O_3 for over 10 h,

2   whereas some MDA production begins with as little as 6 h exposure (Sakaki et al., 1983).

3   Consistent production of MDA, indicative of gross disruptions, occurred only after 8 h exposure

4   (Sakaki et al., 1985), within the timescale when chlorophyll and carotenoid levels began to

5   decline. Concurrently, the total fatty acid (FA) level decreased from ~481 to 358 nmol/cm^2 as

6   the MDA level increased from 0.6 to 2.4 nmol/cm^2, indicating FA peroxidation (Sakati et al.,

7   1985).

8   Sakaki et al. (1983) also studied development of changes by cutting disks from exposed

9   leaves and floating them on water solutions for varied time periods (up to 24 h). This permitted

10   feeding experiments to be done easily, whereas the cutting gives rise to an additional wound

11   response and eliminates metabolite movement to and from other portions of the plant. The

12   floating experiments indicated that, after exposure, scavengers of singlet oxygen ($^1O_2$), such as

13   D_2O, and of hydroxyl radicals, such as benzoate and formate, have no effect on development of

14   the MDA response after 8 h of in vivo fumigation, while scavengers of (O_2^-), such as tiron and

15   ascorbate, lowered the amount of MDA formed. By measuring metabolites immediately after

16   cessation of fumigation, they were able to show that ascorbate loss began with the onset of

17   fumigation, as did SOD loss. A lag time associated with the production of DHA suggested that

18   the reaction of ascorbate with fumigation did not immediately produce the oxidation product.

19   The first 4 h of exposure yielded 30 nmole/cm^2 of ascorbate loss with 5 nmole/cm^2 of DHA

20   production, whereas the second 4 h of exposure yielded 20 nmole/cm^2 of ascorbate loss with

21   20 nmole/cm^2 of DHA production.

22   Nouchi and Toyama (1988) exposed Japanese morning glory (*Ipomea nil*) and kidney bean

23   (*Phaseolus vulgaris*) to 0.15 ppm O_3 for 8 h. Under these conditions, little visible injury was

24   found with up to 4 h exposures, while injury increased by ~50% after 8 h of exposure. Morning

25   glory produced more MDA than kidney bean, which produced the same as the zero-time control.

26   Morning glory also demonstrated a slight (5%) drop in MDGD (monogalactosyldiacylcerol),

27   with increases in PC (phosphatidylcholine), PG (phosohatidylglycerol), PI (phosphatidylinositol),

28   and PE (phosphatidyelthanolamine) after 4 h. Twenty-four hours later, the drop in MGDG

29   (mongalactosyldiacyglyerol) was much larger and was thought to be related to an inhibition of

30   UDP-galactose galactotransferase due to a rise in free fatty acids (FFAs) in the chloroplast. Note

1   that the two distinct timescales involved in O_3 fumigation, immediately post-fumigation and a

2   day or so later, allows for comparison after plant metabolism responds to the fumigation event.

3   The pathway for the formation of MGDG and DGDG (digalactosyldiacylglcerol) is located

4   on the chloroplast envelope. Diacylglycerol (DG) arrives from either the endomembrane system

5   or the stroma and the enzyme UDP-Galactose:1,2-diacylglycerol galactosyltransferase (UDGT)

6   forms MGDG with galactose from UDP-galactose. Sakaki et al. (1990) suggested that the

7   O_3-induced inhibition of UDGT was due to a release of FFAs from within the chloroplast. These

8   FFAs are inhibitory to UDGT, but not to GGGT, which is stimulated by high concentrations of

9   Mg^{2+} (Sakaki et al., 1990). The Sakaki et al. (1990) data indicate that the measured activities of

10   both enzymes isolated after fumigation has indicated that in vivo are not affected by O_3

11   fumigation in vivo. Both enzymes have sensitivity sulfhydryls, and both are located on the

12   envelope. Ozone, if it reaches those sulfhydryls, should inhibit these enzymes; yet inhibition

13   was not seen.

14   It has been thought for years that tocopherols functioned as antioxidants in biological

15   systems (Tappel, 1972). Hausladen et al. (1990) examined the role of antioxidants in red spruce

16   by following seasonal changes. They fit the level of tocopherol within the needles (/g-FW) to

17   the time of the year and found little change (fit as level $= A + Bt + Ct^2$). From this empirical fit,

18   they found that the constant A was lower with higher levels of O_3. The seasonal variation

19   coefficients, B and C, were also lower, suggesting year long lows tocopherol levels. Variation

20   with the season is not particularly surprising, given that phytochrome action may be linked to

21   tocopherol biosynthesis (Lichtenthaler, 1977). Hansladen et al. (1990) reported a significant

22   ($p < 0.05$) trend in the difference between the high and low level of treatment; although there

23   was no discussion of why it occurred or what it meant in relation to metabolism. Their major

24   conclusion was that the antioxidant changes due to O_3 exposure may decrease cold hardiness.

25   Sterols, believed to act as membrane stabilizers, have been investigated by several groups

26   with mixed results. Tomlinson and Rich (1971), who exposed common bean at 0.25 ppm for

27   3 h, and Grunwald and Endress (1985), who exposed soybean at 0.07 ppm for 6 h for 48 days,

28   reported an increase in free sterols and a decline in esterified sterols. However, Trevanthen et al.

29   (1979) exposed tobacco at 0.3 ppm for 6 h and reported opposite results. None of these

30   investigators believed that O_3 had attacked the sterols directly, instead, they believed that these

31   changes involved metabolism and membrane stability. If O_3 induced a metabolic shift that

1 　　disturbed the polar lipid to sterol balance, membrane reactions to other stressors, such as cold

2 　　tolerance, would certainly also be affected, perhaps detrimentally.

3

9.3.8　Role of Age and Size Influencing Response to Ozone

5 　　　　Clearly many changes occur with O_3 exposure can be observed within hours, or perhaps

6 　　days, of the exposure. This document has argued that many of those events are connected with

7 　　wounding and elicitor-induced changes in gene expression, but those are relatively fast acting

8 　　changes (a timescale of tens of hours). Two other effects due to O_3 take longer to occur and tend

9 　　to become most obvious under long periods of low-O_3 concentrations. These have been linked

10 　　to senescence or some other physiological response very closely linked to senescence. These

11 　　two responses, separated by a time sequence, are shown diagrammatically in Figure 9-15.

12

13
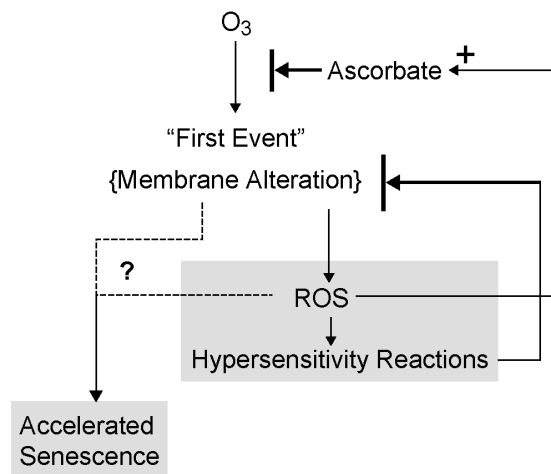
Figure 9-15.　Linkage of senescence with hypersensitivity reactions and first event of O_3 attack of plants.

1 　　　　The understanding of how O_3 affects long-term growth and resistance to other biotic and

2 　　abiotic insults in long-lived trees is unclear. Often, the conditions to which a tree is subjected to

3 　　in one year will affect the response of that tree in the next year. This has been called "memory

4 　　effect", although the term "carry-over" is preferred. In other words, a condition in an earlier

5 　　year sets the stage for a reaction in the next year; thereby giving a "cause-effect" scenario.

1   In perennial plant species, growth affected by a reduction in storage carbohydrates may

2   result in the limitation of growth the following year (carry-over effects). Carry-over effects have

3   been documented in the growth of tree seedlings (Hogsett et al., 1989; Sasek et al., 1991; Temple

4   et al., 1993; U.S. Environmental Protection Agency, 1996) and in roots (Andersen et al., 1991;

5   U.S. Environmental Protection Agency, 1996). Accumulation of the carry-over effects over time

6   will affect survival and reproduction. Data on the cumulative effects of multiple years of O_3

7   exposures have been, for the most part, the result of 2- to 3-year seedling studies. The difficulty

8   of experimentally exposing large trees to O_3 has lead to the tacit assumption that seedling

9   response to O_3 is a good predictor of large-tree response to O_3 (U.S. Environmental Protection

10 Agency, 1996).

11   The carry-over effects of O_3 exposures as observed in tree seedlings cited above by Hogsett

12 et al. (1989) have been termed "memory effects" by Langebartels et al. (1997) and proposed by

13 Schmieden and Wild (1995) to explain the sensitivity of spruce seedlings to frost in the winter

14 after having been exposed to O_3 during the previous summer. Norway spruce exposed to 80 ppb

15 for a whole growing season, demonstrated visible injury symptoms the following year when the

16 new needle flush appeared (Langebartels et al., 1997). Additional studies using Norway spruce

17 and Scots pine seedlings have showed similarly delayed responses following O_3 exposures.

18 Carry-over symptoms were noted to develop at different times of the year, depending on the

19 species of seedling exposed: in early spring for Norway spruce, and in early autumn for Scots

20 pine (Lange et al., 1989). Visible effects of O_3 exposures on spruce and pine may develop after a

21 substantial delay during the "sensitive" periods of the year when chlorophyll and needle loss

22 normally occur. Norway spruce and Scots pine differ in their sensitive periods because of the

23 different needle classes normally remaining on the tree (Langebartels et al., (1997).

24   Nutrient status of the tree during the over-wintering phase of its life (Schmieden and Wild,

25 1995) and chronic exposure to ambient O_3 (less severe with fewer peaks of very high levels)

26 induce (1) mineral nutrient deficiency; (2) alterations of normal metabolism, including

27 translocation and allocation of carbohydrates and probably nitrogen; and (3) disturbance of

28 normal transpiration and diurnal cycling, leading to water stress. This condition, termed

29 "Montane yellowing", appears to be related to nutrient deficiencies rather than senescence

30 (although early loss of leaves and needles does occur). While generalized low nutrient

31 concentrations may not occur within the foliage, localized deficiencies may. However, they are

1   hard to observe or prove without a great deal of work involving all portions of a tree and without

2   a general hypothesis of what is occurring.

3

4   9.3.9 Summary

5   As the understanding of wounding responses of plants and more genome details and varied

6   plant mutants become available, the cellular and physiological responses of plants to O_3

7   exposures are slowly becoming clearer. However, more studies are needed on a larger variety of

8   species. Nevertheless, several key findings and conclusions can be highlighted:

9   (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".

10   (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.

11   (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.

12   (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.

13   (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.

14   It is important to note that the dramatic strides in understanding the genetic makeup of

15   plants, gene control, and signal transduction/control over the last few years will likely accelerate

16   in the future. That understanding will translate into better models of the hypotheses listed above

17   and more detailed schemes of how O_3 alters much of basic plant metabolism. Thus, while

18   understanding of how O_3 interacts with the plant at a cellular level has dramatically improved,

19   the translation of those mechanisms into how O_3 is involved with altered cell metabolism, with

20   whole plant productivity, and with other physiological facts remained to be more fully

21   elucidated.

22

23

24   **9.4   MODIFICATION OF FUNCTIONAL AND GROWTH RESPONSES**

25   **9.4.1   Introduction**

26   The responses of plants to any air pollutant may be significantly influenced by a wide

27   range of biological, chemical and physical factors. A plant's genetic make-up is an important

28   inherent biological determinant of its response, but response can also be modified by other

29   biological agents such as disease-causing organisms, insects and other pests, and by other higher

30   plant species with which it may be competing for resources. Chemical factors that may

31   influence response range from mineral nutrients obtained from the soil to other air pollutants and

32   agricultural chemicals. Physical factors that may influence response include light, temperature

33   and the availability of moisture, which are components of climate and climate change.

34   Some environmental factors are capable of being controlled, to some degree, by man,

35   while others are not. The biological factors (pests, diseases, symbioses and competition) are

36   partly controllable in agriculture but much less so (if at all) in natural ecosystems. It is possible

37   to control agricultural soil fertility and the use of agricultural chemicals, as well as to exercise

38   some control over the supply of water and airborne chemical factors. In contrast, the physical

39   factors, i.e., light and temperature, are uncontrolled in the field even though they may be

40   controllable in specialized situations such as greenhouses or shade houses.

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

12   In view of the large number of factors to be considered and given that the purpose of this
13   document is to support the review of the O_3 NAAQS, including standards to protect vegetation,
14   this section focuses mainly on situations in which there is clear evidence that environmental
15   factors truly interact with oxidant effects, i.e., they magnify or diminish the impact of O_3 and are
16   *not merely additive* to it. Conversely, it will cover situations where O_3 acts synergistically or
17   antagonistically, but not additively, with effects induced by other factors. It will also emphasize
18   those interactions as a result of which overall plant growth and development, and yield are
19   adversely affected, rather than the details of interactions at the mechanistic level, unless the latter
20   are deemed to be essential to an understanding of larger scale effects.

21   To facilitate cross-reference, the present document uses essentially the same subsections as
22   in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). Although light and
23   temperature are components of climate, they are initially reviewed as individual physical factors,
24   even though temperature effects are revisited to some extent in the discussion of interactions
25   with climate change.

26   Few studies reported since the 1996 document have systematically investigated
27   quantitative responses to O_3 concurrently with other variables. Although the 1996 document
28   cited almost 300 references pertaining to environmental interactions, and the present review cites
29   more than 350 new references, the bulk of the recently published work has continued to be
30   determined by the specific and frequently narrowly focused interests of individual researchers or
31   groups. Hence, the new findings are scattered and far from uniformly distributed among the

1   various sub-topics. In some instances there has been little or no research published that adds to

2   our understanding since the 1996 document. In such cases, the present review is therefore

3   restricted to summarizing the understanding that was current in 1996.

4   A few reviews have appeared since the early 1990s dealing with various environmental

5   interactions, and these are cited in relevant sections below. More general recent reviews are

6   those of Wellburn (1994); multi-authored volumes edited by Alscher and Wellburn (1994),

7   Yunus and Iqbal (1996), De Kok and Stulen (1998), and Bell and Treshow (2002); and reports

8   by the United Nations Environment Programme (UNEP) (1999) and the Intergovernmental

9   Panel on Climate Change (IPCC) (2001). Several biotic and abiotic interactions involving forest

10   trees are discussed in the review by Johnson et al. (1996b).

11   Although many reports have provided quantitative information on interactive effects, in

12   most cases the information is only descriptive of a specific situation involving only two or three

13   levels of a variable. While this may be adequate to provide statistical information about the

14   existence of interactions with environmental factors, it does not permit the development of

15   response surfaces or models to show the form that any influence of such factors might take on

16   O_3 exposure-response relationships or how O_3 might quantitatively influence responses to the

17   factors in question. This, together with the fragmented information available on the effects of

18   most factors, has contributed to the relative lack of development of simulation models of

19   oxidant-environmental factor interactions. Yet, as noted by Taylor et al. (1994), the large

20   number of variables constrains the assessment of pollution effects by experimentation alone.

21   The only alternative is to use mathematical models to attempt to predict the outcome of different

22   O_3 and environmental factor scenarios, building up their complexity in stages. The few models

23   thus far used to investigate O_3 stress have been adapted from existing process models of crop or

24   tree growth which include limited numbers of physical or chemical variables (such as

25   temperature, soil water stress, or nutrient deficiency). Taylor et al. (1994) provide a listing of

26   several simulation models developed for trees at the individual, stand, and regional levels, and

27   these and many other models have been critically reviewed by Kickert and Krupa (1991) and

28   Kickert et al. (1999). However, regardless of whether such models are descriptive/empirical or

29   process/mechanistic, their outputs will always be associated with varying degrees of uncertainty

30   and require validation against observable responses wherever possible. Kickert et al. (1999) also

31   point out that very few of the models that have been described provide *risk assessments* that

1   address likelihood, in contrast to *consequence assessments* that address the magnitudes of

2   effects. Thus, even though capable simulation models of plant response to O_3 involving

3   complex mixes of many biological, physical and chemical factors may be out of reach at the

4   present time, the use of newer mathematical approaches such as artificial neural networks

5   (ANNs) has enabled insightful analyses to be performed in several field studies involving

6   numerous micrometeorological and other environmental variables (e.g., Balls et al., 1996;

7   Mills et al., 2000).

8   Because the ensuing subsections deal with studies of O_3 interactions involving an

9   extremely wide array of biological, physical, and chemical factors in the plant's environment,

10 it is inevitable that many different exposure facilities and regimes have been used in these

11 studies. To provide specific information regarding the O_3 exposure concentrations, profiles,

12 hours and days of exposure (as well as the types of systems and facilities used for the exposures)

13 would add a wealth of detail that would do little to assist our understanding of the roles of

14 environment factors in modifying the impact of O_3 on vegetation or to facilitate our ability to

15 estimate the magnitudes of any such modifications. Thus, only experiments in which the

16 exposure levels and regimes were *within the bounds of ambient experience in North America*

17 are discussed in the ensuing subsections, regardless of the type of exposure profile used. The

18 cutoffs used have been: ~200 ppb for peak hourly concentrations or for short-term exposures;

19 ~100 ppb for daytime means involving prolonged exposures for several hours; or a doubling of

20 ambient levels, in cases in which enriched exposure levels were a function of ambient levels.

21 Actual details of the exposure regimes and conditions can, of course, be obtained from the

22 original references but are only stated here when any distinction needs to be made between the

23 effects of different exposure levels. Hence, it should be understood that ensuing statements such

24 as "... it was found that O_3 caused ..." should always be read as "... it was found that exposures to

25 O_3 (within the range of those that have been measured in ambient air) caused ..."

26

27 **9.4.2 Genetics**

28   The response of individual plants to O_3 is affected by several factors including the

29 environment in which it is growing, competition with neighbor plants, ontogeny, and genetics.

30 This section examines the role of genetics in plant response to O_3. In addition, major knowledge

31 gaps in the understanding of genetic aspects of O_3 response are pointed out.

1   It is well known that species vary greatly in their responsiveness to O_3 (Environmental

2   Protection Agency, 1996). This again has been recently demonstrated for grassland species

3   (Warwick and Taylor, 1995; Pleijel and Danielson, 1997; Grime et al., 1997; Bungener et al.,

4   1999a, b; Franzaring et al., 2000), wild herbaceous plants (Bergmann et al., 1999; Danielsson

5   et al., 1999; Nussbaum et al., 2001), agricultural crops (Renaud et al., 1997; Ollerenshaw et al.,

6   1999; Benton et al., 2000; Heagle and Stefanski, 2000; Fumagalli et al., 2001; Elagöz et al.,

7   2002; Nali et al., 2002; Köllner and Krause, 2003), horticultural shrubs and trees (Hormaza

8   et al., 1996; Findley et al., 1997), and forest trees (Pääkkönen et al., 1997; Volin et al., 1998; Pell

9   et al., 1999; Bortier et al., 2000; Postiglione et al., 2000; Landolt et al., 2000; Zhang et al., 2001;

10   Oksanen and Rousi, 2001; Matsumura, 2001; Guidi et al., 2001; Momen et al., 2002; Nali et al.,

11   2002; Saitanis and Karandinos, 2002). These studies have shown a wide range of responses to

12   O_3, from growth stimulation by a few species such as *Festuca ovina* L. (Pleijel and Danielson,

13   1997) and *Silene dioica* and *Chrysanthemum leucanthemum* (Bungener et al., 1999) to

14   significant growth reduction, depending on environmental conditions and exposure dose.

15   While determining the explanation for differences in species sensitivity to O_3 remains one

16   of the challenges facing plant biologists (Pell et al., 1999), a number of hypotheses have been

17   suggested. Reich (1987) proposed that variation in O_3 sensitivity could be explained by variation

18   in total uptake of the gas. Others have suggested that (1) fast-growing species are more sensitive

19   than slower growing ones (Bortier et al., 2000), (2) overall O_3 sensitivity may be closely linked

20   to root responses to O_3 (Warwick and Taylor, 1995) or (3) the relative ability of species to

21   detoxify O_3-generated reactive oxygen free radicals may determine O_3 sensitivity (Alscher et al.,

22   1997; Pell et al., 1999). Volin et al., (1998) suggest that the relative rate of stomatal

23   conductance and the photosynthesis rate at a given conductance both contribute strongly to

24   determining species sensitivity to O_3. Likely, there is more than one mechanism determining

25   sensitivity, even in a single species.

26   Within a given species, individual genotypes or populations can also vary significantly in

27   O_3 sensitivity (Environmental Protection Agency, 1996). For example, the intraspecific

28   variation in O_3 sensitivity for was a factor of two for *Phleum pratense* (Danielsson et al., 1999)

29   and *Trifolium repens* L. (Postiglione et al., 2000). A similar range of intraspecific variations in

30   O_3 responses was demonstrated for clonal differences in *Betula pendula* by Pääkkönen et al.

31   (1997) and *Prunus serotina* (Lee et al., 2002). These examples of wide ranges within species

1 responses suffice to show that caution should be taken when ranking species categorically as

2 having an absolute degree of O_3 sensitivity (Davison and Barnes, 1998).

3

4 **9.4.2.1 Genetic Basis of O_3 Sensitivity**

5   Plant response to ozone is determined by genes that are directly related to oxidant stress

6 and to an unknown number of genes that are not specifically related to oxidants. The latter

7 includes genes that control leaf and cell wall thickness, stomatal conductance and the internal

8 architecture of the air spaces. Although there is currently a great emphasis on individual

9 antioxidants that can be manipulated by molecular methods, the challenge is to determine the

10 relative contributions of all of the components to plant response and to understand the interplay

11 between them. Recent studies using molecular biological tools are beginning to increase the

12 understanding of O_3 toxicity and differences in O_3 sensitivity.

13   While much of the research in developing the understanding of O_3 responses has been

14 correlative in nature, recent studies with transgenic plants have begun to positively verify the

15 role of various genes and gene products in O_3 tolerance. Overexpressing MnSOD in chloroplasts

16 increased O_3 tolerance in transgenic tobacco plants (Van Camp et al., 1994) provided the first

17 definitive proof of antioxidants key role in O_3 tolerance. Subsequently, Broadbent et al. (1995)

18 showed that overexpression of pea glutathione reductase simultaneously in both chloroplasts and

19 mitochondria of transgenic tobacco enhanced O_3 tolerance. Similarly, increased O_3 tolerance to

20 O_3-induced foliar necrosis was shown for transgenic tobacco plants overexpressing the cytosolic

21 Cu/Zn-SOD gene (Pitcher and Zilinskas, 1996). Transgenic tobacco plants expressing antisense

22 RNA for cytosolic ascorbate peroxidase, which reduces ascorbate peroxidase production,

23 showed increased susceptibility to O_3 injury suggesting a key role in O_3 tolerance for the

24 antioxidant ascorbate peroxidase (Örvar and Ellis, 1997).

25   The consensus among molecular studies of O_3 sensitivity is pointing to O_3 triggering

26 salicylic acid, ethylene, and jasmonic acid and that the signaling of these molecules determines,

27 in some cases, the O_3 susceptibility of plants (Overmyer et al., 2000; Rao and Davis, 1999; Rao

28 et al., 2000; Langerbartels et al., 2002; Moeder et al., 2002; Tamaoki *et al.,* 2003; Vahala et al.,

29 2003a, b). Increased levels of jasmonic acid production in O_3-tolerant compared to O_3-sensitive

30 plants has been shown for *Arabidopsis* (Overmyer et al., 2000) and *Populus* (Koch et al., 1998,

31 2000). Blockage of ethylene production by using antisense methods with ACC synthase and

1   ACC oxidase suggest strongly that ethylene synthesis and perception are required for H_2O_2

2   production and cell death following O_3 exposure of *Lycopersicon esculentum* (Moeder et al.,

3   2002). Ethylene signaling may have multiple roles in O_3 tolerance determination as was

4   demonstrated recently by Vahala et al., (2003a, b) who found that in *Populus tremula* ×

5   *P. tremuloides* hybrid clones differing in O_3 sensitivity, ethylene accelerated leaf senescence in

6   sensitive plants under low O_3, but under acute O_3, ethylene seemed to be required for protection

7   from cell death.

8   While changing expressions of single antioxidant genes has proven very useful in

9   identifying possible mechanisms of O_3 sensitivity and tolerance (Kuzniak, 2002), it should be

10   noted that some studies of transgenic plants with enhanced antioxidant production have not

11   resulted in increased O_3 tolerance (Saji et al., 1997; Torsethaugen et al., 1997; Strohm et al.,

12   1999, 2002). Clearly, ethylene production plays a role in O_3 sensitivity but the role of various

13   antioxidants in O_3 tolerance regulation are yet to be fully elucidated (Wellburn and Wellburn,

14   1996). It is unlikely that single genes are responsible for O_3 tolerance responses, except in rare

15   exceptions (Engle and Gabelman, 1966). Regulation of stomatal opening and leaf structure

16   (Bennett et al., 1992; Elegoz and Manning, 2002) are also likely to play key roles in O_3 tolerance

17   in plants. Newly developing opportunities to examine simultaneous regulation of larger numbers

18   of genes will likely yield more clarification of genes controlling O_3 tolerance (Desikan et al.,

19   2001; Matsuyama et al., 2002).

20   Attempts to demonstrate conclusively changes in antioxidant and protective pigments for

21   O_3 sensitive and tolerant mature trees growing in the field have largely been unsuccessful (Tausz

22   et al., 1999a, b). However, evidence for antioxidant expression differences contributing to

23   differences in O_3 sensitivity of four-year-old *Populus tremuloides* trees has been found

24   (Wustman et al., 2001).

25

26   ## 9.4.3 Environmental Biological Factors

27   The biological factors within the plant's environment that may influence its response to O_3

28   directly or may be influenced to the advantage or disadvantage of plants encompass insects and

29   other animal pests, diseases, weeds and other competing plant species. Although such

30   interactions are ecological in nature, those involving individual pests, plant pathogens, or weeds,

1   or agricultural crop or forest tree species are considered in this section. More complex

2   ecological inter-species interactions are dealt with in Section 9.5.

3   The different types of biological factors are dealt with separately, as in the 1996 O_3 AQCD

4   (U.S. Environmental Protection Agency, 1996). Still, it is important to recognize certain general

5   features of relationships of plants with biological components of their environment:

6   • Successful infestation or infection involves complex interactions among the target or host
     species, the causal organism and environmental factors.

7   • Infestations and infections may co-occur.

8   • The successful development and spread of a pest, pathogen or weed require favorable
     environmental factors.

9   • Significant losses to crops and forest trees result from pests and pathogens.

10   • Significant losses to crops and seedling trees result from weed competition.

11   Ozone and other photochemical oxidants may influence the severity of a disease or

12   infestation by a pest or weed, either by direct effects on the causal species, or indirectly by

13   affecting the host, or both. In addition, the interaction between O_3, a plant and a pest, pathogen

14   or weed may influence the response of the target host species to O_3. A perceptive overview of

15   the possible interactions of O_3-exposure with insect pests and fungal diseases has been provided

16   by Jones et al. (1994), based on a model system involving two insects and two pathogens

17   affecting cottonwood (*Populus deltoides*). Their study also included effects on the

18   decomposition of leaf litter.

19   In contrast to detrimental biological interactions, there are mutually beneficial relationships

20   or symbioses involving higher plants and bacteria or fungi. These include (1) the nitrogen-fixing

21   species *Rhizobium* and *Frankia* that nodulate the roots of legumes and alder, and (2) the

22   mycorrhizae that infect the roots of many crop and tree species, all of which may be affected by

23   exposure of the host plants to O_3.

24   In addition to the interactions involving animal pests, O_3 may also have indirect effects on

25   higher herbivorous animals, e.g., livestock, due to O_3-induced changes in feed quality.

26

27   **9.4.3.1   Oxidant-Plant-Insect Interactions**

28   The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) stressed the variability

29   in the reported effects of O_3 on host plant-insect interactions. Since relatively few plant-insect

1 systems have been studied, few consistent patterns of response have emerged, as noted in other

2 reviews such as those of Colls and Unsworth (1992), Heliövaara and Väisänen (1993), Whittaker

3 (1994), Docherty et al. (1997) and, most recently, Flückiger et al. (2002).

4   None of the studies reported in the past decade have clarified the situation in terms of

5 clearly consistent effects. A 1997 review by Docherty et al. (1997), for example, examined

6 17 reports of studies of aphid species on a range of hosts and classed the O_3 effects on aphid

7 performance as: 35% positive; 41% negative; and 24% showing no significant effect.

8 A tabulation of 19 studies by Flückiger et al. (2002) gave the corresponding figures: 42%,

9 21%, and 37%.

10   Other recent studies with the aphids *Schizolachnus pineti* and *Cinara pinea* on Scots pine

11 (*Pinus sylvestris*) and *Cinara pilicornis* on Norway spruce *(Picea abies)* have also yielded

12 variable results, with them suggesting that O_3 enhances aphid density on pine and aphid

13 performance on spruce (Holopainen et al., 1997; Kainulainen et al., 2000a). In an earlier study

14 with *Schizolachnus pineti* on Scots pine, Kainulainen et al. (1994) had observed no significant

15 effects of O_3-treatment on aphid performance. However, more recent observations of long-term

16 effects on aphid populations on aspen (*Populus tremuloides*) exposed to O_3 in a FACE system

17 revealed that O_3 significantly increased aphid populations and decreased the populations of

18 predatory insects (Percy et al., 2002).

19   The observations of Brown et al. (1993) and Jackson (1995) led Whittaker (1994) and

20 Brown (1995) to suggest that aphid response was dependent on ambient temperature as well as

21 the dynamics of O_3 exposure; growth tended to be stimulated with maximum temperatures below

22 ~20 °C but was reduced at higher temperatures. The present situation with plant-aphid responses

23 therefore remains confused and, although numerous suggestions have been offered to explain

24 specific findings, they are difficult to assemble into a coherent picture.

25   Variability has also been found with the interactions involving chewing insects. For

26 example, Lindroth et al. (1993) reported a small negative O_3 effect (8% reduction) on the growth

27 of gypsy moth larvae (*Limantra dispar*) on hybrid poplar (*Populus tristis × balsamifera*) but no

28 effect when on sugar maple (*Acer saccharum*). Ozone resulted in reduced growth rate of the

29 larvae of the bug *Lygus rugulipennis* on Scots pine, but enhanced the growth of larvae of the

30 sawfly *Gilpinia pallida* (Manninen et al., 2000). Costa et al. (2001) observed no significant O_3

1   effects on the growth and fecundity of the Colorado potato beetle (*Leptinotarsa decemlincata*)

2   on potato in greenhouse and field experiments.

3   Fortin et al. (1997), in a two-year study of forest tent caterpillar (*Malacosoma disstria*) on

4   sugar maple, observed that O_3 exposure only increased the growth rate of female larvae in one

5   year; 4th- and 5th-instar larvae also showed a feeding preference for treated foliage in that year.

6   However, studies based on open-air exposures of aspen (*Populus tremuloides*) indicated

7   O_3-enhanced growth of *M. disstria* in terms of pupal weight (Percy et al., 2002) and larval

8   performance (Kopper and Lindroth, 2003). Jackson et al. (2000) observed inconsistency in

9   studies on the larva of the tobacco hornworm (*Manduca sexta*) on tobacco (*Nicotiana tabacum*).

10   In one year, feeding on O_3-treated foliage resulted in significantly greater larval weight, whereas

11   in a second year the increase was not statistically significant although survival was increased.

12   Also, oviposition by hornworm moths was increased if ambient O_3 levels were increased by 70%

13   but fell back to normal in ambient O_3 levels (Jackson et al., 1999).

14   Studies of the two-spotted spider mite (*Tetranychus urticae*) on white clover (*Trifolium*

15   *repens*) and peanut (*Arachis hypogeae*) by Heagle et al. (1994) and Hummel et al. (1998)

16   showed that, on peanut and an O_3-sensitive clover clone, O_3-exposure stimulated mite

17   populations. The lack of significant effects on mites on the O_3-resistant clover clone suggests

18   that the responses were host-mediated.

19   With chewing insects and mites, there therefore appears to be a clearer indication of the

20   likelihood that increased insect performance will result from O_3-induced changes in the host

21   plant, but negative effects continue to be reported, indicating that the response is probably also

22   being determined in part by other environmental, genetic, or temporal variables.

23   Reported O_3-induced enhancement of attack by bark beetles (*Dendroctonus brevicomis*) on

24   Ponderosa pine (*Pinus ponderosa*) has been suggested by Dahlsten et al. (1997) to be due to

25   greater brood development on injured trees, possibly related to decreased numbers of predators

26   and parasitoids. This view gains some support from the observation that O_3 adversely affected

27   the searching behavior of a parasitoid, *Asobara tabida*, for larvae of *Drosophila subobscura*

28   which led to fewer parasitized hosts (Gate et al., 1995). Such observations reveal another level

29   of complexity in the O_3-plant-insect interrelationship: O_3 may reduce the effectiveness of the

30   natural control of insect pests. The phenomenon is probably related to effects on olfactory cues,

1 since it was shown by Arndt (1995) that O$_3$ can affect fly behavior by modifying the pheromones

2 that cause fly aggregation.

3   These reports focus on the direct or indirect effects on the insect or mite feeding on foliage

4 previously or currently exposed to O$_3$. They provide little if any information on effects on the

5 host plant other than qualitative references to the injury caused by the O$_3$-exposure. Enhanced

6 pest development will ultimately lead to increased adverse effects on the hosts in the long term,

7 but the only report of an O$_3$-plant-insect interaction directly affecting the host plant in the short

8 term still appears to be that of Rosen and Runeckles (1976). They found that infestation by the

9 greenhouse whitefly (*Trialeurodes vaporariorum*) sensitized bean plants (*Phaseolus vulgaris*) to

10 injury by otherwise non-injurious low levels of O$_3$, leading to premature senescence of the

11 leaves.

12   The overall picture regarding possible O$_3$ effects on plant-insect relations, therefore,

13 continues to be far from clear. Only a few of the very large number of such interactions that

14 may affect crops, forest trees and other natural vegetation have been studied. The trend

15 suggested in the 1996 criteria document that O$_3$ may enhance insect attack has received some

16 support from a few recent studies. However, the variability noted in most of the studies makes it

17 clear that we are still far from being able to predict the nature of any particular O$_3$-plant-insect

18 interaction or its magnitude or severity.

19

20 **9.4.3.2 Oxidant-Plant-Pathogen Interactions**

21   Plant diseases are caused by pathogenic organisms, e.g., fungi, bacteria, mycoplasmas,

22 viruses, and nematodes. Ozone impacts on disease are briefly discussed in earlier reviews by

23 Ayres (1991) and Colls and Unsworth (1992) and, more recently, by Flückiger et al. (2002).

24 Biotic interactions with forest trees have been reviewed by Chappelka and Samuelson (1998);

25 and Sandermann (1996) and Schraudner et al. (1996) have summarized molecular similarities

26 and interrelationships between necrotic O$_3$ injury to leaves and pathogen attack. A few recent

27 publications have added to our fragmented knowledge of O$_3$-plant-disease interactions and the

28 mechanisms involved, but there appear to have been no reports to date of studies involving

29 mycoplasmal diseases.

30   The 1996 criteria document (U.S. Environmental Protection Agency, 1996) noted the

31 concept put forward by Dowding, (1988) "that pathogens and pests which can benefit from

1   damaged host cells and from disordered transport mechanisms are enhanced by pollution insult

2   to their hosts, whereas those pathogens and other symbionts which require a healthy mature host

3   for successful invasion are depressed by pollutant stress to their host." The pathogens of the first

4   type are mostly facultative, necrotrophic, fungal parasites, whereas the second type are largely

5   obligate biotrophic fungi, bacteria and viruses. Based on this distinction, the majority of the

6   cases cited in the 1996 document supported Dowding's view, as have several more recent studies

7   summarized in Table 9-11. However, there are also some contradictions.

8   Most investigations have focused on the incidence and development of disease on plants

9   previously or concurrently exposed to O_3, rather than on the corollary effect of disease on the

10   response to O_3. In all of the studies of facultative pathogens and the nematode studies, exposure

11   to O_3 tended to result in increased disease severity through increased spore germination or

12   increased fungal growth and development, although in the case of grey mold (*Botrytis cinerea*)

13   on kidney bean (*Phaseolus vulgaris*) this was only observed on an O_3-sensitive cultivar

14   inoculated with conidia (Tonneijck, 1994). Using mycelial inoculation, O_3 reduced disease

15   development, but no satisfactory explanation was offered to account for the difference in

16   response. With leaf spot, *Marssonina tremulae*, on hybrid poplar (*Populus trichocarpa* ×

17   *balsamifera*), low level exposures to O_3 also increased disease (in agreement with theory) but

18   higher levels (200 ppb, 8h per day for 15 days) reduced conidial germination (Beare et al.,

19   1999b).

20   The situation with obligate biotrophic pathogens is less consistent. The effects on powdery

21   mildew (*Sphaerotheca fulginea*) on both bottle gourd (*Lagenaria siceraria*) and cucumber

22   (*Cucumis sativa*) resembled the situation with the necrotrophic poplar leaf spot disease

23   (*Marssonina tremulae*), since low O_3 exposures increased disease severity (in disagreement with

24   theory) although higher levels decreased it. The decreased infection in the pea-powdery mildew

25   (*Erysiphe polygoni*) situation agrees with theory, but the situations with leaf rust (*Melampsora*

26   sp.) on poplar (*Populus trichocarpa* × *balsamifera*) or aspen (*Populus tremuloides*) do not.

27   However, these reports are in contrast to earlier reports included in the 1996 criteria document

28   (U.S. Environmental Protection Agency, 1996) of observations with other species of *Erysiphe*

29   (Tiedemann et al., 1991) and *Melampsora* (Coleman et al., 1987). In contrast to the recent report

30   of a synergism with *Melampsora* on poplar, infections caused by the other biotrophs

31   (*Sphaerotheca, Erysiphe, Uromyces*) reduced the severity of injury caused by O_3 (in agreement

Table 9-11. 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 9-11 (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, 1998a)

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

1 with numerous earlier reports), but only at high O_3 exposures in the case of *Sphaerotheca* on

2 cucumber. At low exposure levels, the disease and O_3 acted synergistically. The only other

3 recent observation of such disease-related synergisms are the nematode-tomato reports of (Khan

4 and Khan, 1997, 1998b).

5   It is therefore clear that the type and magnitude of exposure to O_3 plays an important role

6 in determining both the response of the disease organism and of the host.

7   No recent studies involving interactions between O_3 and bacterial diseases appear to have

8 been reported. With regard to viruses, a laboratory study by Yalpani et al. (1994) added to

9 several reports of O_3 decreasing the severity of tobacco mosaic virus infection of tobacco,

10 *Nicotiana tabacum*; and Jimenez et al. (2001) reported that previous exposure to O_3 resulted in

11 increased adverse effects on tomato yield attributed to several virus diseases.

12   Similarities between the sensitivities of different cultivars or clones to O_3 and to specific

13 diseases have been noted. For example, Sah et al. (1993) found that the severity of injury caused

14 by tar spot and standard O_3 exposures of 12 wheat cultivars were closely correlated ($R^2 = 0.986$).

15 Such similarities appear to have a mechanistic basis, since several studies have noted similarities

16 in the molecular and biochemical changes that occur in plants infected with pathogens and plants

17 exposed to O_3. Schraudner et al. (1992), Ernst et al. (1992), Eckey-Kaltenbach et al. (1994a,b),

18 Yalpani et al. (1994) and Bahl et al. (1995) have presented evidence that exposures to O_3 result

19 in responses such as increased levels of salicylic acid, the signaling agent for increased induced

20 resistance to pathogens. This, in turn, leads to activation of the genes that encode defense

21 proteins, including the so-called pathogenesis-related proteins. The induction of such proteins

22 might account for the decreased infection with *Sphaerotheca* and *Melampsora* at higher O_3

23 exposures but does not account for increased infections seen at lower exposure levels. The issue

24 is discussed more fully by Sandermann (1996) and Schraudner et al. (1996). More recently

25 Sandermann has extended the theory relating O_3 and disease by suggesting that, because of O_3

26 "memory effects" in affected host plants that may persist over weeks or months, analysis for

27 various induced biomarkers of gene activation may provide a useful tool for improving our

28 ability to predict the outcome of O_3-plant-pathogen interactions (Sandermann, Jr., 2000).

29   There have been no reports of O_3 studies with mixed infections by pathogens, but the

30 complete suppression of *Heterobasidion* butt and root rot of Scots pine by the mycorrhizal

1   symbiont *Hebeloma crustuliniforme* indicates the possibility of interactions involving more than

2   one fungus (see Section 9.4.4.3.3 below).

3   In summary, our understanding of oxidant-plant-disease interactions is far from complete.

4   However, a combined tabulation of the evidence presented in the 1996 O_3 AQCD (U.S.

5   Environmental Protection Agency, 1996) and that noted in Table 9-11 leads to the following

6   summary of O_3 effects on plant diseases and corollary effects of infection on plant response

7   to O_3, as indicated by number of studies showing increases or decreases in disease or

8   susceptibility:

9   For obligate biotrophic fungi, bacteria, nematodes:

10   O_3 increased disease: 9   Increased susceptibility to O_3:   3.

11   O_3 decreased disease: 15   Decreased susceptibility to O_3:   9.

12   For facultative necrotrophic fungi:

13   O_3 increased disease: 25   Increased susceptibility to O_3:   2.

14   O_3 decreased disease: 3   Decreased susceptibility to O_3:   4.

15 Thus, although O_3 may reduce the severity but not the incidence of some of the diseases caused

16 by the obligate pathogens, the evidence overall indicates that with most diseases, their severity is

17 more likely to be increased by O_3 than not. However, the actual consequences will be specific to

18 the disease and level of exposure, and, most importantly, will be determined by environmental

19 suitability and epidemiological requirements for disease to develop. Conversely, some evidence

20 suggests that infection by obligate pathogens may confer some degree of "protection" against O_3,

21 a dubious benefit from the plant's point of view.

22

23 **9.4.3.3   Oxidant-Plant-Symbiont Interactions**

24   No further studies have appeared regarding O_3 effects on the important bacterial symbiont

25 of legumes, *Rhizobium*, since those summarized in the 1996 document (U.S. Environmental

26 Protection Agency, 1996). Hence, our present understanding is that, although relatively high

27 levels of exposure (> 200 ppb) can result in severe (> 40%) reductions in nodulation (and

28 therefore nitrogen-fixation) on soybean roots, lesser reductions in nitrogen-fixing may be caused

29 by lower exposures. However, the data are inadequate to attempt to define any quantitative

30 exposure-response relationships.

1   There have been a few recent reports on O_3-plant-mycorrhizae interrelationships. These

2   have mostly involved seedlings of coniferous tree species. A transient O_3-induced stimulation of

3   mycorrhiza on Scots pine roots reported by Kasurinen et al. (1999) was not observed in a later

4   study by Kainulainen et al. (2000b). Studies of the mycorrhiza *Paxillus involutus* on birch

5   (*Betula pendula*) seedlings showed that, although O_3 reduced mycorrhizal growth rate, it led to

6   greater extension growth which in turn resulted in greater mycorrhizal infection of neighboring

7   Aleppo pine (*Pinus halepensis*) seedlings (Kytöviita et al., 1999). However, O_3 reduced nitrogen

8   acquisition by *P. halepensis* from its mycorrhizal symbiont (Kytöviita et al., 2001). The

9   complex interrelationships that may occur in the rhizosphere were revealed by the observation

10   (Bonello et al., 1993) that the mycorrhiza *Hebeloma crustuliniforme* could overcome the

11   O_3-stimulated severity of root rot on Scots pine (*Pinus sylvestris*) caused by the fungus

12   *Heterobasidion annosum* (noted in Section 9.4.4.3.2).

13   In summary, the available evidence is far too fragmented and contradictory to permit

14   drawing any general conclusions about mycorrhizal impacts. The negative effects of O_3 on

15   mycorrhizae and their functioning that have been reported have not necessarily been found to

16   lead to deleterious effects on the growth of host plants. Thus, little has changed from 1991 when

17   Dighton and Jansen asked: "Atmospheric Pollutants and Ectomycorrhizae: More Questions

18   than Answers?" (Dighton and Jansen, 1991). Because of their important roles in ecosystems,

19   mycorrhizae are further discussed in Section 9.5 below.

20

21   **9.4.3.4 Oxidant-Plant-Plant Interactions: Competition**

22   Plant competition involves the ability of individual plants to acquire the environmental

23   resources needed for growth and development: light, water, nutrients and space. Intraspecific

24   competition involves individuals of the same species, typically in monocultural crop situations,

25   while interspecific competition refers to the interference exerted by individuals of different

26   species on each other when they are in a mixed culture.

27   In cropping situations, optimal cultural practices for row spacing and plant density/row

28   tend to balance the negative effects of intraspecific competition and the goal of maximum yield.

29   Although interspecific competition is agriculturally undesirable when it involves weak

30   infestations, the use of mixed plantings may be agriculturally deliberate, e.g., grass-legume

1   mixtures used for pasture or forage. In natural plant communities, monocultures are rare, and
2   complex interspecific competition is the norm.

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

8   The tendency for O_3-exposure to shift the biomass of grass-legume mixtures in favor of
9   grass species, reported in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) has
10   been confirmed by recent studies. In a ryegrass (*Lolium perenne*) + clover (*Trifolium repens*)
11   mixture grown in an open-air fumigation system, clover growth was impaired by extended
12   exposures to above-ambient O_3, leaving patches for weed invasion (Wilbourn et al., 1995).
13   An open-top chamber study by Nussbaum et al. (1995b) using the same species confirmed the
14   greater effect on clover but observed that the magnitude of the effect depended highly on the
15   pattern of O_3-exposures over extended growing periods. Low-level exposures shifted species
16   composition in favor of *Lolium*, but exposures to higher peak O_3 levels depressed total mixture
17   yield. With an alfalfa (*Medicago sativa*) + timothy (*Phleum pratense*) mixture, Johnson et al.
18   (1996a) noted that O_3 caused decreased alfalfa root growth and increased timothy shoot growth
19   and height. Nussbaum et al. (2000a) reported that, with increased exposure to O_3, well-watered
20   red clover (*Trifolium pratense*) plants suffered from increased competition from the grass,
21   *Trisetum flavescens*, but the O_3 exposure also negatively effected grass growth, depressing
22   overall total yield. However, a greater adverse effect on *Trisetum* resulted from O_3-induced
23   increased competition when grown with brown knapweed (*Centaurea jacea*), a weed species.

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

29   Three studies have been reported on more complex plant associations. Ashmore and
30   Ainsworth (1995) studied mixed plantings of two grasses, *Agrostis capilaris* and *Festuca rubra*,

1 with two forbs[16], *Trifolium repens* (a legume) and *Veronica chamaedrys*, exposed to O_3 in

2 open-top chambers (OTCs). The proportion of forbs, *Trifolium* in particular, declined, especially

3 when cut at biweekly intervals. In a related study, Ashmore et al. (1995) used artificial mixtures

4 of grasses and forbs and transplanted swards of native calcareous grassland species and found

5 that, regardless of whether total biomass was adversely affected by exposures to O_3, higher

6 exposures progressively shifted species composition, usually at the expense of the forb species.

7 The observed shifts in competitive balance in favor of grasses is consistent with observations

8 that many grass species are less sensitive to O_3 than forbs. However, as previously shown by

9 Evans and Ashmore (1992), knowledge of the relative sensitivities to O_3 of the component

10 species grown in isolation or in monoculture does not always predict the impact of O_3 on the

11 components in a mixed culture.

12   Barbo et al. (1998) exposed an early successional plant community to O_3 in OTCs for two

13 growing seasons. Ozone decreased community structure features such as height of canopy,

14 vertical canopy density (layers of foliage), and species diversity and evenness. Surprisingly,

15 blackberry (*Rubus cuneifolius*), a species considered to be O_3-sensitive, replaced sumac (*Rhus*

16 *copallina*) canopy dominance. Barbo et al. (2002) also demonstrated the role of competition in

17 determining the impact of O_3 on loblolly pine (*Pinus taeda*). They reported that the increased

18 growth of natural competitors in OTCs using charcoal-treated air to reduce the ambient O_3

19 concentrations resulted in decreased pine growth. They noted that this is contrary to the

20 frequently reported increased growth observed in reduced O_3 levels in the absence of

21 interspecific competition.

22   Competitively disadvantaged trees of four clones of aspen (*Populus tremuloides*) exposed

23 to 1.5× ambient O_3 in a FACE facility over 4 years were proportionately more adversely affected

24 by O_3 than competitively advantaged or neutral trees (McDonald et al., 2002). However, one

25 clone of the disadvantaged trees demonstrated enhanced growth.

26   In summary, our present knowledge of how O_3 may affect the competitive interspecific

27 plant-plant relationships typifying the agricultural and natural worlds is very limited. However,

28 as noted in the 1996 AQCD (U.S. Environmental Protection Agency, 1996), "the development

29 and use of field exposure systems have permitted many recent studies of crop species to be

30 conducted at normal planting densities and hence have incorporated intraspecific competition as

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

1   an environmental factor." Such facilities were used in most of the studies of interspecific

2   competition discussed above. But we are still a long way from being able to bridge the gap

3   between small model competing systems and the realities of natural ecosystem complexity.

4

9.4.4 Physical Factors

6   The physical features of a plant's aerial and edaphic environments exercise numerous

7   controls over its growth and development. Thus, many of their effects may be modified by

8   exposure to atmospheric oxidants and, alternatively, plants may modify responses to such

9   exposures. As in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), this

10   section focuses on the defining features of plant microclimate: light, temperature, relative

11   humidity (HR, or saturation vapor pressure deficit), and the presence and availability of water,

12   especially in the soil. Monteith and Elston (1993) suggested that light energy and mass of water

13   should be viewed as climatic resources, and the other two elements (temperature and saturation

14   vapor pressure deficit) as rate modifiers that determine how fast the resources are used. The

15   modifications of plant response by physical environmental factors has recently been reviewed by

16   Mills (2002).

17   Another physical feature of the microclimate, wind and air turbulence, which affects the

18   thicknesses of the boundary layers over leaves and canopies and, hence, affects gas exchange

19   rates (including the fluxes of O_3 and other oxidants into the leaves) is discussed elsewhere

20   (Section 9.3).

21   Physical features of the environment are also important components of larger-scale

22   regional and global climates. However, the following discussions are confined to issues related

23   to individual factors at the plant level; meso-scale effects are reviewed in Section 9.4.4.8, which

24   addresses the issues of climate change interactions.

25

9.4.4.1 Light

27   Plants are the primary producers of biomass on the planet through their ability to capture

28   light energy (by the process of photosynthesis) and convert it to the many forms of chemical

29   energy that sustain their own growth and that of secondary consumers and decomposers. Light

30   *intensity* is critical since the availability of light energy (a resource, *sensu* [Monteith and Elston,

31   1993]) governs the rate at which photosynthesis can occur, while light *duration* (i.e.,

1   photoperiod) profoundly effects development in many species. Although light *quality* (i.e., the

2   distribution of incident wavelengths) may also affect some physiological plants processes, there

3   is no evidence to indicate that such effects are of relevance to concerns over oxidant pollution,

4   except at the short wavelengths of UV-B. This topic is discussed in the context of climate

5   change in Section 9.4.8, and as a stress factor per se affected by atmospheric O_3 in Chapter 8.

6   However, as noted above and in the 1996 O_3 AQCD (U.S. Environmental Protection Agency,

7   1996), none of the features is controllable in natural field situations. A brief discussion of light

8   intensity-O_3 interactions is included in the review by Chappelka and Samuelson (1998).

9   The conclusion in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) that

10   low light intensities and short photoperiods tended to increase susceptibility to foliar O_3-injury

11   may still be valid, but this may or may not translate into adverse effects on growth. For

12   example, Tjoelker et al. (1993) found that, when seedlings of sugar maple (*Acer saccharum*),

13   a shade-tolerant species, were grown in 7% full sunlight, O_3 reduced shoot and root growth, but

14   had no significant effect in 45% sunlight (a 6-fold increase). In contrast, the reverse was

15   observed with a shade-intolerant hybrid poplar (*Populus tristis × balsamifera*), with the greater

16   impact of O_3 occurring in the higher light intensity treatment.

17   The greater sensitivity of maple in low light has also been confirmed in other studies.

18   Tjoelker et al. (1995) noted a greater O_3-induced inhibition of photosynthetic CO_2 assimilation in

19   shaded leaves than in leaves in full sunlight. However, in the absence of differences in stomatal

20   conductance, the effect was considered to be independent of O_3 flux; it appeared to be a

21   consequence of reduced chlorophyll contents and quantum efficiencies induced by O_3.

22   In contrast, Bäck et al. (1999), who also observed a greater inhibition of net photosynthesis by

23   O_3 in shaded leaves, reported decreased stomatal conductance. Although reduced conductance

24   might suggest reduced O_3 flux and, therefore, decreased adverse effects, the authors concluded

25   that the effects of reduced conductance was offset by long-term changes in leaf structure, leading

26   to less densely packed mesophyll cells and greater internal air space within the leaves.

27   Morphological differences between lower and upper crown leaves of black cherry (*Prunus*

28   *serotina*) have been suggested as the basis for the greater O_3-susceptibility of the lower crown

29   leaves (Fredericksen et al., 1995). Bäck et al. (1999) also observed accelerated foliar senescence

30   induced by O_3 on shaded leaves, a response also noted by Topa et al. (2001). Sensitivity to O_3

31   was found to be increased in shade- but not sun-leaves of shade-tolerant red oak (*Quercus rubra*)

1   (Samuelson and Edwards, 1993). Similarly, Mortensen (1999) observed that seedlings of

2   mountain birch (*Betula pubescens*) grown in 50% shade suffered greater foliar injury from O_3

3   than those grown in full sunlight.

4   Not all shade-intolerant species exhibit greater reductions in photosynthesis and growth

5   due to O_3 when grown in full sunlight. Higher than ambient levels of O_3 failed to inhibit

6   photosynthesis in leaves of shade-intolerant yellow poplar (*Liriodendron tulipifera*) grown in

7   nearly full sunlight (Tjoelker and Luxmoore, 1991). Greater foliar injury in the lower, shaded

8   leaves of shade-intolerant black cherry (*Prunus serotina*) trees and saplings, was attributed

9   (Fredericksen et al., 1996a) to higher stomatal conductance and greater O_3 uptake relative to net

10 photosynthetic rate. However, in a 3-year study of Norway spruce seedlings (*Picea abies*) in

11 OTCs. Wallin et al. (1992) observed that photosynthetic efficiency was more adversely affected

12 by O_3 in high than in low light.

13   The suggestion of greater sensitivity to O_3 of shade-tolerant species in low-light conditions

14 and the greater sensitivity of shade-intolerant species in high light is somewhat of an over-

15 simplification when dealing with mature trees, for which light intensity varies considerably

16 within the canopy because of shading. Chappelka and Samuelson (1998) noted that the

17 interaction between sensitivity to O_3 and the light environment in forest trees is further

18 complicated by developmental stage, with seedlings, saplings, and mature trees frequently giving

19 different results. Topa et al. (2001) also cautioned that O_3 effects on leaf-level photosynthesis

20 may be poor predictors of the growth responses of sugar maple in different light environments.

21   In high-light intensities, many species exhibit some degree of photoinhibition of the

22 photosynthetic process through the overloading of the mechanisms that protect the

23 photosynthetic reaction centers in the chloroplasts. Guidi et al. (2000) reported complex

24 interactions between high-light intensities (inducing photoinhibition) and O_3 exposures in kidney

25 bean (*Phaseolus vulgaris*) with high intensities tending to enhance the detrimental effect of O_3

26 on photosynthesis. One of the studies in the extensive European Stress Physiology and Climate

27 Experiment-wheat (ESPACE-wheat) program (Bender et al., 1999), conducted in 1994 and

28 1995, included an investigation of the effects of climatic variables on yield response to O_3 using

29 two simulation models, AFRCWHEAT2-O_3 and LINTULCC (Ewart et al., 1999; Van Oijen and

30 Ewart, 1999). Among the observed trends, it was noted that relative yield loss of wheat due to

31 elevated O_3 tended to increase with light intensity. In contrast, Balls et al. (1996) used ANNS to

1   investigate microclimatic influences on injury caused by O_3 to clover (*Trifolium subterraneum*)

2   and found that, especially at mid-range cumulative O_3 exposures (350 to 500 ppb-h), injury

3   tended to decrease with increasing light intensity. Similar observations by Davison et al. (2003)

4   of foliar injury to wild populations of cutleaf cone flower (*Rudbeckia laciniata*) exhibiting a

5   range of PAR levels within their canopies led the authors to conclude that the variation in injury

6   symptoms observed was "unlikely to be due to differences in ozone flux and more likely to be

7   due to variation in light." Antonielli et al. (1997) found evidence indicating that the high

8   sensitivity of the bioindicator tobacco cultivar *Nicotiana tabacum* cv Bel-W3 is partly

9   determined by its high photosynthetic electron transport rates at high-light intensities, which

10   exceed the capabilities of the plant to dissipate energy and oxyradicals.

11   The 1996 O_3 AQCD referred to the important role of light in controlling stomatal opening

12   and suggested that light duration (i.e., photoperiod) might dictate the actual uptake of O_3 to some

13   degree. However, it should also be noted that Sild et al. (1999) found that clover plants could

14   suffer foliar injury even if they were exposed to O_3 during the dark period of the day-night cycle,

15   when stomatal conductance is at its lowest.

16   A possible indirect effect of light intensity was noted by Reiling and Davison (1992) in

17   their study of the O_3-tolerance of *Plantago major* plants grown from seeds collected from

18   populations at 28 different sites in Britain. Ozone-tolerance, defined in terms of plant growth,

19   was found to be a function of both previous O_3-exposure history and hours of bright sunshine

20   during the year before the seeds were collected. However, the authors cautioned that, since

21   tropospheric O_3-formation is itself dependent upon irradiation, the observation does not

22   necessarily imply a direct effect of light intensity on the plants' response to O_3.

23   The only recent studies concerning interactions with light quality appear to be those

24   involving O_3 and UV-B as a component of climate change. These are dealt with in Section

25   9.4.4.8.2. The effects of photoperiod on response to O_3 or the converse do not appear to have

26   received any recent attention.

27   Although the intensity, quality, and duration of light are not controllable in the natural

28   world, the interactions of O_3 with light intensity, in particular, clearly have relevance to the

29   growth of shade-tolerant and shade-intolerant species in mixed forest stands. It appears that the

30   nature of light intensity-O_3 interactions may depend upon the type of light environment to which

31   the species are best adapted, with increased light intensity increasing the sensitivity of light-

1   tolerant and decreasing the sensitivity of shade-tolerant species to O_3. Although there is

2   certainly some evidence to the contrary, this hypothesis is a reasonable summation of current

3   understanding with regard to O_3-light intensity interactions.

4

5   **9.4.4.2   Temperature**

6   "Temperature determines the start and finish and rate and duration of organ growth and

7   development" (Lawlor, 1998). Such processes depend on fundamental physiological activities

8   that are mostly enzyme-mediated and whose kinetics are directly affected by temperature. Since

9   the processes of enzyme deactivation and protein denaturation also increase as temperatures rise,

10   each enzymatic process has a unique optimum temperature range for maximal function.

11   However, the optima for different processes within the plant vary appreciably and, hence, the

12   optimum temperature range for overall plant growth is one within which all of the individual

13   reactions and vital processes are *collectively functioning optimally,* not necessarily *maximally*.

14   Furthermore, individual features of plant development (e.g., shoot and root growth, flowering,

15   pollen tube growth, fruit set, seed development) have different specific optima, so that

16   differential responses to temperature occur, leading to temperature-induced developmental

17   changes. For example, despite increased assimilation, increased temperatures may result in

18   decreased grain yields of crops such as wheat, because the growing season is effectively

19   shortened by a more rapid onset of senescence (Van Oijen and Ewart, 1999).

20   Rowland-Bamford (2000) noted that a plant's response to temperature changes will depend

21   upon whether it is growing at its near optimum temperature for growth or its near maximum

22   temperature, and whether any increase in mean temperature results in temperatures rising above

23   the threshold for beneficial responses. Impairment by O_3 of any process may be thought of as

24   being analogous to a downward shift below and away from the temperature optimum or an

25   upward shift above and away from the optimum. Since a temperature rise toward the optimum

26   would result in a rate increase, the combined effects of O_3 and such an increase might neutralize

27   each other, while the effects of O_3 and a decrease in temperature would likely be additively

28   negative. Above the optimum temperature, the situations would be reversed with the effects of

29   increased temperatures and O_3 being additively negative, and decreasing temperatures

30   counteracting any negative effect of O_3. Thus, it is difficult to generalize about the interactions

31   of temperature and O_3 on overall plant responses such as growth in which the different

1   temperature-rate relationships of different growth components are merged, because they depend

2   upon the relationship of any temperature changes to the optimum for a species.

3   Studies of the effects of temperature on the impact of O_3 have increased recently because

4   of an increased need to understand the consequences of global warming as a component of

5   climate change. Direct interactions of temperature with O_3 are reviewed here, but the issues are

6   addressed again in Section 9.4.8.1 in relation to changes in atmospheric CO_2 levels.

7   The 1996 criteria document (U.S. Environmental Protection Agency, 1996) stressed the

8   interdependence of the temperature within the tissues of the leaf (where the various temperature-

9   sensitive processes occur) on three distinct components: the ambient air temperature, the heating

10 effect of incident infrared radiation during the photoperiod, and the evaporative cooling effect

11 caused by transpirational loss of water. It also cautioned that, especially in experiments using

12 controlled environment chambers, the effects of temperature could well be confounded with

13 those of humidity/vapor pressure deficit (VPD). Temperature and VPD are strongly interrelated,

14 and VPD plays an important role in regulating stomatal transpiration. Because of the role that

15 evaporative cooling plays in determining internal leaf temperatures, any factor that causes

16 stomatal closure and reduced conductance inevitably leads to increased leaf temperatures. Such

17 interactions add to the difficulties in distinguishing the effects of temperature from those of other

18 factors, as actual leaf temperatures are rarely measured and reported.

19   Despite these caveats, there is some evidence that temperature per se influences plant

20 response to O_3. For example, in rapid-cycling Brassica (*Brassica rapa*) and radish (*Raphanus*

21 *sativus*), marked O_3-inhibitions of growth were observed at low root temperatures (13 °C) but

22 not at 18°C (Kleier et al., 1998, 2001). With regard to air temperature, this was included in the

23 range of micrometeorological variables studied in several recent extensive field studies and was

24 found to have a significant effect on response to O_3 in most cases. Ball et al. (1996) used ANNs

25 in an analysis of the growth of clover (*Trifolium subterraneum*) and concluded that light and

26 VPD had greater influences than temperature on the visible injury response to O_3. However, in

27 three studies with different cultivars of white clover (*Trifolium repens*), temperature was found

28 to be important to the growth response. Ball et al. (1998) exposed *T. repens* cv. Menna to

29 ambient O_3 in OTCs at 12 European sites at a range of latitudes and altitudes from 1994 to 1996.

30 The impact of O_3 on growth was determined as the ratio of growth with and without treatment

31 with the O_3-protectant, EDU (see Section 9.2). Artificial neural network analysis showed that O_3

1   exposure (measured as the AOT40 index, see Section 9.4.6), VPD, and temperature were

2   consistently the three most important variables governing response to O_3 over a range of

3   different ANN models. However, the authors did not describe the form of the O_3-response

4   relationship with temperature. Similar observations were reported by Ball et al. (2000) and Mills

5   et al. (2000) for O_3-sensitive and O_3-tolerant clones of *T. repens* cv. Regal, grown at 14 to

6   18 European locations from 1995 to 1998. In both studies, the impact of O_3 was measured as the

7   sensitive/tolerant growth (biomass) ratio. Although Ball et al. (2000) found temperature to be

8   less important than O_3 exposure and VPD, Mills et al. (2000) found temperature to be the most

9   important input variable after O_3 exposure (AOT40). In both cases, the adverse effect of O_3

10   increased with increasing temperature.

11   A study of black cherry (*Prunus serotina*) seedlings and mature trees in Pennsylvania,

12   using micrometeorological variables aimed to predict O_3 uptake, found temperature to be

13   unimportant (Fredericksen et al., 1996b), but in the study of populations of *Plantago major*

14   referred to in Section 9.4.4.1, Reiling and Davison (1992) noted a weak, positive correlation

15   between mean temperature at the collection site and O_3 tolerance (based on growth rate) of the

16   different populations. In contrast, Danielsson et al. (1999) collected genotypes of *Phleum*

17   *arvense* from a wide range of Nordic locations and found a positive effect of temperature on the

18   growth of genotypes from locations wth higher summer temperatures, but sensitivity to O_3 did

19   not vary systematically with geographic location.

20   Van Oijen and Ewart (1999) studied the effects of climatic variables on the response to O_3

21   in the ESPACE-wheat program, based on two distinctive simulation models (AFRCWHEAT2-

22   O_3 and LINTULCC, [Ewart et al., 1999]) and noted that although the relative yield loss of wheat

23   due to elevated O_3 tended to increase with temperature, the effect was of minor significance.

24   In contrast to the variable results obtained in studies of the effects of temperature on

25   response to O_3, the corollary effect of O_3 exposure on subsequent sensitivity to low temperature

26   stress, noted in the 1996 criteria document, is well recognized. In reviewing low temperature-O_3

27   interactions, Colls and Unsworth (1992) noted that winter conditions produce three kinds of

28   stress: desiccation, chilling or freezing temperatures, and photooxidation of pigments. Of these,

29   they suggested that while the first two were important, the last may play a particularly significant

30   role because the "combination of high irradiance and low temperatures permits a buildup of free

31   radicals in leaf tissue, and these free radicals then attack chlorophyll." Chappelka and Freer-

1   Smith (1995) suggested that the injury and losses to trees caused by this delayed impact of O_3

2   may be equally or more important than the direct impacts of O_3 on foliage of visible injury and

3   necrosis, or the disruption of key physiological processes such as photosynthesis. In this

4   context, the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) referred to the

5   conceptual framework (Eamus and Murray, 1991), which is still valid: brief periods of mild

6   temperatures in the severest winters result in dehardening; O_3 decreases frost hardiness per se,

7   but also increases the predisposition to dehardening; dehardening places O_3-exposed trees at

8   greater risk from subsequent low temperatures. However, no quantified models of these effects

9   have yet appeared.

10   The 1996 criteria document also noted that O_3 adversely affects cold hardiness of

11   herbaceous species. More recently, Foot et al. (1996, 1997) observed winter injury and

12   decreased growth in low-growing perennial heather *Calluna vulgaris* exposed to O_3 (70 ppb,

13   8 h/day, 5 days/week for 6 months) during the winter (6.8 °C mean), but found no significant

14   effects of the same exposures during the summer (12.3 °C mean). Although Potter et al. (1996)

15   observed a similar situation with the moss *Polytrichum commune*, the reverse was found with the

16   moss *Sphagnum recurvum*.

17   In summary, unequivocal evidence exists that O_3 causes sensitization to the adverse effect

18   of low temperatures, but there is no clear pattern to evidence regarding the effects of temperature

19   on O_3 response. The many contradictory responses to temperature and O_3 probably reflect our

20   lack of detailed knowledge of the temperature optima for the different growth components of the

21   studied species. The topic of temperature-oxidant interactions is revisited later in Section 9.4.4.8

22   in the context of global warming as a feature of climate change.

23

24   **9.4.4.3   Humidity and Surface Wetness**

25   The moisture content of the ambient air (or its VPD) is a rate modifier (*sensu* Monteith and

26   Elston, 1993) and an environmental regulator of stomatal conductance. Both of the previous

27   criteria documents (U.S. Environmental Protection Agency, 1986, 1996) concluded that the

28   weight of evidence indicated that high RH (= low VPD) tended to increase the adverse effects of

29   O_3, principally because the stomatal closure induced in most situations by O_3 is inhibited by high

30   RH, leading to increased O_3 flux into the leaves.

1   Recent reports have confirmed this role of RH. The studies by Ball et al. (1995, 1996,

2   1998) showed that VPD was an important determinant of O_3-induced injury and reduced growth

3   in two species of clover, *Trifolium repens* cv. Menna and *T. subterraneum*. However, Mills et al.

4   (2000) found it to be unimportant in the case of *T. repens* cv. Regal. Such difference between

5   cultivars is not unexpected since considerable differences also occur among species and genera.

6   For example, Bungener et al. (1999a) studied 26 Swiss grassland species and found clear

7   evidence that O_3 injury increased with decreased VPD (i.e., increased RH) in only eight species.

8   However, the 1995 data from the European cooperative study of O_3 injury, which involved

9   28 sites in 15 countries and six crop species, led to the development of two 5-day critical-level

10   scenarios involving O_3-exposure (calculated as the AOT40 index) and mean VPD (0930-1630h):

11   200 ppb-h at > 1.5 kPa, and 500 ppb-h at < 0.6 kPa (Benton et al., 2000).

12   With forest tree species, Fredericksen et al. (1996b) found significant correlations between

13   stomatal conductance of black cherry (*Prunus serotina*) leaves and RH (+ve) and VPD (−ve),

14   and studies on free-standing Norway spruce (*Picea abies*) and larch (*Larix decidua*) showed that

15   although ambient VPD was highly positively correlated with ambient O_3 concentration,

16   increased VPD caused stomatal closure, reducing O_3 uptake and impact (Wieser and Havranek,

17   1993, 1995).

18   Surface wetness may affect the response to O_3 through its direct effects on deposition to

19   the surface and through changes in RH. Effects on the deposition of O_3 have been reviewed

20   (Cape, 1996). A surface film of water on leaves was found to increase O_3 deposition in four

21   studies involving field-grown grape (*Vitis vinifera*) (Grantz et al., 1995), red maple (*Acer*

22   *rubrum*) (Fuentes and Gillespie, 1992), deciduous forest dominated by largetooth aspen (*Populus*

23   *grandidentata*) and red maple (Fuentes et al., 1992), and clover-grass mixed pasture (*Trifolium*

24   *pratense*, *Phleum pratense*, and *Festuca pratensis*) (Pleijel et al., 1995). In each case, the

25   increased deposition could be attributed partly to an increased stomatal conductance through the

26   abaxial (lower) surface and partly to uptake into the aqueous film on the adaxial (upper) surface.

27   In contrast, decreased deposition was noted by Grantz et al. (1997) with field-grown cotton

28   (*Gossypium hirsutum*). Since cotton is amphistomatous, with functional stomata on both leaf

29   surfaces, it was suggested that, in this case, the water layer effectively sealed the adaxial surface

30   stomata, more than offseting any increase in conductivity of the stomata in the abaxial surface.

31   However, none of the studies investigated the consequences of the differences in deposition.

1　Although it could be inferred that, with part of any increased deposition being the result of

2　increased O_3 flux into the leaves, there would be the likelihood of increased O_3 adverse effects,

3　as suggested by earlier studies (Elkiey and Ormrod, 1981) that, by misting bluegrass (*Poa*

4　*pratensis*) during exposure to O_3, injury was significantly increased.

5　　　To conclude, the effects of high RH (low VPD) and surface wetness have much in

6　common, as they both tend to enhance the uptake of O_3, largely through effects on stomata,

7　leading to increased impact.

8

9　**9.4.4.4　Drought and Salinity**

10　　　The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) concluded that the

11　available evidence clearly indicated that exposure to drought conditions could reduce the adverse

12　effects of O_3 on the growth of herbaceous and woody plants, but it also noted that no quantitative

13　models of the O_3-soil moisture deficit (SMD) interaction had yet appeared in print.

14　Nevertheless, the "protective" effect was inconsistent, and only appeared when SMD was

15　accompanied by high evaporative demand. Since that time, further studies have confirmed the

16　interaction, and simulation models have begun to appear. Mills (2002) has recently provided a

17　brief review of the topic.

18　　　With regard to herbaceous species, Vozzo et al. (1995) observed less O_3-induced injury and

19　suppression of net photosynthesis and growth in water-deficient soybean (*Glycine max*) than in

20　well-watered plants. In several studies with wheat (*Triticum aestivum*), on the other hand,

21　although adverse effects of both O_3 and SMD were noted, they were consistently additive

22　(Bender et al., 1999; Fangmeier et al., 1994b, 1994a; Ommen et al., 1999).

23　　　In attempting to model the stomatal conductance of wheat in relation to O_3 and soil

24　moisture, Grüters et al. (1995) found that although O_3-induced stomatal closure was enhanced by

25　SMD, reducing O_3 uptake, the R^2 of the overall model was only 0.40, indicating that other

26　significant factors or relationships were involved.

27　　　With regard to native vegetation, Bungener et al. (1999a) used mixed plantings of 24 Swiss

28　grasses, herbs, and legumes and observed that, although O_3-drought interactions were species-

29　specific, they tended to reflect stomatal functioning. They found that SMD reduced O_3 injury in

30　two clovers (*Trifolium repens* and *T. pratensis*) and two grasses (*Trisetum flavescens* and

31　*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. 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 (*Pinus sylvestris*), by Karlsson et al. (2002) on Norway spruce (*Picea abies*), or by Pelloux et al. (2001) on Aleppo pine (*P. halepensis*). 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 (*P. ponderosa*), and Van den Driessche et al. (1994) reported that drought reduced injury and O_3-induced ethylene release by *Picea abies*. 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. (2002) 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 *Picea abies* in the third year of a 4-year study. A similar response was noted by Dixon et al. (1998) with the Istebna strain of *P. abies.*

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

1   O₃-induced growth reduction. Although European beech (*Fagus sylvatica*) is reportedly an

2   O₃-and drought-sensitive species, neither Pearson and Mansfield (1994) nor Broadmeadow et al.

3   (1999) observed any interactions between these stresses, while Dixon et al. (1998) observed

4   partial protection. Pääkönen et al. (1998) observed only additive effects in a sensitive clone of

5   birch (*Betula pendula*). However, the experiments of Schaub et al. (2003) and the survey by

6   Vollenweider et al. (2003) on black cherry (*Prunus serotina*) cleary indicate antagonism between

7   drought and O₃ stresses on this species.

8   With regard to the converse effect, in a critical review of the evidence for predisposition to

9   drought stress being caused by O₃, Maier-Maercker (1998) supported the hypothesis and

10   suggested that the effects were caused by the direct effects of O₃ on the walls of the stomatal

11   guard and subsidiary cells in the leaf epidermis, leading to stomatal dysfunction.

12   The Plant Growth Stress Model (PGSW) developed by Chen et al. (1994) is a physiology-

13   based process model which includes drought among several environmental variables.

14   Simulations for Ponderosa pine (*Pinus ponderosa*) incorporated antagonistic effects between O₃

15   and drought stresses, i.e. partial protection, although Karlsson et al. (2000) have since

16   emphasized that drought-induced "memory effects" should be considered when developing

17   simulation models incorporating stomatal conductance.

18   Retzlaff et al. (2000) used the single-tree model, TREEGRO, to simulate the combined

19   effects of O₃ and drought on white fir (*Abies concolor*). Although simulated reductions in

20   precipitation ≥ 25% reduced growth, they also reduced O₃ uptake (and impact). But lesser

21   reductions in precipitation combined synergistically with O₃ stress to reduce growth, leading the

22   authors to conclude that moderate drought may not ameliorate the response of white fir to O₃.

23   On a much larger scale with a modified forest ecosystem model (PnEt-II) incorporating O₃-

24   response relationships for hardwood species, Ollinger et al. (1997) showed how predicted

25   changes in net primary production and mean wood production in the northeastern U.S. hardwood

26   forests due to O₃ would be reduced (but not countered or reversed) by drought stress, particularly

27   in the southern part of the region. This geographic distribution of the effect was substantiated by

28   the work of Lefohn et al. (1997) on the risk to forest trees in the southern Appalachian

29   Mountains, based on localized estimates of O₃ levels and SMD. The TREEGRO and ZELIG

30   models were combined by Laurence et al. (2001) to predict the impacts of O₃ and moisture (as

31   precipitation) on the growth of loblolly pine (*Pinus taeda*) and yellow poplar (*Liriodendron*

1 *tulipifera*). Based on O_3 and precipitation data from three sites in the eastern United States, the

2 six model regressions developed for the two species included both positive and negative

3 coefficients for O_3 exposure and precipitation as determinants of growth.

4   As noted in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), the effects

5 of soil salinity are similar to those of SMD. In a study of rice (*Oryza sativa*) cultivars of

6 differing sensitivity to salinity, Welfare et al. (1996) noted that although both O_3 and salinity

7 reduced many features of growth additively, antagonistic interactions were only seen for leaf

8 length and potassium accumulation. Similarly, a recent study on chickpea (*Cicer arietinum*)

9 found no interactions with regard to most components of biomass accumulation (the effects

10 of O_3 and salinity were additive), but with root growth, salinity suppressed the adverse effects

11 of O_3.

12   In summary, the recently described interactions of O_3 and drought/salinity stresses are

13 consistent with the view that, in many species, drought/salinity reduces the impact of O_3 but O_3

14 increases sensitivity to drought stress, i.e., the type of response is determined by the sequence of

15 stresses. However, synergisms have also been observed and any antagonisms are species-

16 specific and unpredictable in the absence of experimental evidence. In no case has an

17 antagonism been found to provide complete protection.

18

9.4.5 Nutritional Factors

20   The 1996 criteria document (U.S. Environmental Protection Agency, 1996) noted that the

21 large number of macro- and micronutrients and the wide range of species had prevented

22 experimental investigation of all but a few cases of nutrient-O_3 interactions and most of these

23 concerned nitrogen (N) and crops or forest tree species. The document also provided a

24 comprehensive tabulation of the results of the relevant studies up to 1992.

25   The suboptimal supply of mineral nutrients to plants leads to various types of growth

26 reductions. The consequences of suboptimal nutrition might, therefore, be expected to have

27 some similarities to those of O_3 exposure. One might expect nutritional levels below the

28 optimum either to amplify any effects of O_3 or at least lead to additive responses. The difficulty

29 with this suggestion is that the available information has mostly been obtained from

30 experimentation conducted using two or more arbitrarily selected levels of fertility with little or

31 no regard to optima. Hence, it is not surprising that there have been contradictory reports, even

1   among studies with the same species or cultivars conducted by different workers at different

2   locations using different soils or soil mixes.

3   There appear to have been no recent studies on O_3 interactions with specific mineral

4   nutrients other than N. Hence, the previous conclusions are still valid, viz. that increasing levels

5   of the major elements potassium (K) and sulfur (S) usually reduce the impact of O_3, or,

6   deficiency increases susceptibility, whereas increased phosphorus (P) usually increases injury,

7   or, deficiency decreases susceptibility.

8   However, with N, a relationship to the optimum is usually demonstrable. Several earlier

9   studies of $O_3 \times$ N interactions reported that the adverse effects of O_3 on growth were greatest at

10   the optimum and decreased with increasing N-deficiency, a finding supported by the work on

11   aspen (*Populus tremuloides*) of Pell et al. (1995), who also confirmed that excess N decreased O_3

12   impact on growth. Similarly, the adverse effects of O_3 on growth rate in wheat (*Triticum*

13   *aestivum*) diminished with decreased N supply (Cardoso-Vilhena and Barnes, 2001). However,

14   the effects of N are far from consistent. For example, Greitner et al. (1994) reported that O_3 and

15   N-deficiency acted additively in aspen in reducing leaf surface area and rate of photosynthesis,

16   Bielenberg et al. (2001) reported that the rate of O_3-induced senescence was increased by

17   N-deficiency in hybrid poplar (*Populus trichocarpa × maximovizii*), and Pääkkönen and

18   Holopainen (1995) observed the least adverse effects of O_3 on birch (*Betula pendula*) at

19   optimum N-fertility levels. With cotton (*Gossypium hirsutum*), increased N-levels more than

20   overcame the adverse effect of O_3 on growth and boll yield (Heagle et al., 1999). In view of

21   these contradictions, one may conclude that other, unrecorded factors may have contributed to

22   the various findings. Thus, much remains unclear about $O_3 \times$ N-fertility interactions.

23   There have been two recent studies on the effects of overall soil fertility. Whitfield et al.

24   (1998) observed that low general fertility increased O_3 sensitivity in selections of *Plantago*

25   *major*. At the biochemical level, well-fertilized birch (*Betula pendula*) saplings were found to

26   be less adversely affected by O_3 than nutrient-stressed plants (Landolt et al., 1997).

27   TREEGRO model simulations of the growth of red spruce (*Picea rubra*) in conditions of

28   nutrient deficiency and O_3 stress showed that, in combination, the two stresses acted less than

29   additively (Weinstein and Yanai, 1994). Minimal amelioration by nutrient deficiency was

30   predicted with Ponderosa pine (*Pinus ponderosa*).

1   Plants may also obtain N and S from airborne sources such as NO_x, HNO_3, NO_3^-, SO_2, and

2   SO_4^{2-}, although, depending upon their concentration, these may also be phytotoxic. In various

3   parts of the world, the deposition of N and S in these forms contributes significantly to the levels

4   of nutritionally available N and S in soils. Such depositions may, in turn, influence the impact of

5   O_3 on sensitive species through their roles as nutrients independent of any interactions that may

6   occur because of their acidic properties (see Section 9.4.6.5). For example, Takemoto et al.

7   (2001) recently reviewed the situation in southern California's mixed conifer forests and noted

8   that, where N-deposition is appreciable, its combination with O_3 is causing a shift in Ponderosa

9   pine (*Pinus ponderosa*) biomass allocation towards that of deciduous trees, with increased

10   needle drop so that only 1- and 2-year needle classes overwinter. Such changes are having

11   significant consequences to the balance of the forest ecosystem and are discussed more fully in

12   Section 9.5.

13   Of the micronutrient elements, only manganese (Mn) appears to have been studied

14   recently. In beans (*Phaseolus vulgaris*) Mn-deficiency increased O_3 toxicity, despite causing

15   reduced O_3 uptake (through decreased stomatal conductance) and inducing increased levels of

16   Mn-SOD (Mehlhorn and Wenzel, 1996).

17   In view of the foregoing, it is impossible to generalize about the interactions of soil fertility

18   with O_3. While this is especially true of the interactions involving soil nitrogen, for which there

19   is much conflicting evidence, the interactions with other nutrients need much more thorough

20   investigation than has occurred to date, before any clear patterns become apparent.

21

22   ## 9.4.6 Interactions with Other Pollutants

23   The ambient air may be polluted by gases other than O_3 and its photochemical oxidant

24   relatives. In particular, industrial, domestic, and automobile emissions and accidents can lead to

25   significant atmospheric concentrations of gases such as sulfur dioxide (SO_2), nitric oxide (NO),

26   and nitrogen dioxide (NO_2), collectively referred to as NO_x, both locally and regionally. Local

27   releases of gases such as hydrogen fluoride (HF), hydrogen chloride (HCl), and chlorine (Cl_2)

28   may result from industrial emissions and accidents. Agricultural fertilizer and manure usage can

29   lead to significant increases in ambient ammonia (NH_3). The sulfur and nitrogen oxides may

30   undergo reactions in the atmosphere leading to the formation of sulphate (SO_4^{2-}) and nitrate

31   (NO_3^-) ions and resultant acid deposition.

1   The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) discounted much of

2   the early research on pollutant combinations, because of its lack of resemblance to the ambient

3   experience: the concentrations used were unrealistically high or the exposure regimes employed

4   almost invariably used gas mixtures, whereas Lefohn et al. (1987) showed that the co-occurrence

5   patterns of significant levels of O_3 with SO_2 or NO_2 in the United States were most frequently

6   sequential or partially sequential with overlap; only rarely were they entirely concurrent. On the

7   other hand, O_3 and peroxyacetylnitrate (PAN) frequently co-occur, as both form photochemically

8   under similar conditions.

9   To the list of reviews mentioned in the 1996 criteria document should be added the more

10   recent ones by Barnes and Wellburn (1998), Robinson et al. (1998), and Fangmeier et al. (2002),

11   which also explore some of the potential mechanisms underlying pollutant-pollutant interactions.

12

13   **9.4.6.1   Oxidant Mixtures**

14   In 1998, Barnes and Wellburn noted that virtually no information existed on the effects on

15   plants of concurrent exposures to O_3 and other components of photochemical oxidant other than

16   PAN. The situation has not changed since their review appeared, and the topic appears to have

17   attracted no research interest since before the 1996 O_3 AQCD (U.S. Environmental Protection

18   Agency, 1996). The continuing conclusion must, therefore, be that, from the limited information

19   available, the two gases appear to act antagonistically, with O_3 raising the threshold for the

20   visible injury response to PAN, and PAN reducing the harmful effects of O_3.

21

22   **9.4.6.2   Sulfur Dioxide**

23   In reviewing $O_3 \times SO_2$ interactions, Barnes and Wellburn (1998) remarked: "The outcome

24   of exposure to this combination of pollutants has probably been the most studied, yet is one of

25   the least understood." More recent studies have only added to the conflicts referred to in the

26   1996 criteria document (U.S. Environmental Protection Agency, 1996), rather than resolve them.

27   For example, Diaz et al. (1996) reported that, after a year of daily exposures of Aleppo pine

28   (*Pinus halepensis*)seedlings to 50 ppb O_3 and/or 40 ppb SO_2, the combination of pollutants

29   synergistically reduced shoot and root growth and impaired mycorrhizal colonization of the

30   roots. With tomato (*Lycopersicon esculentum*), on the other hand, effects on growth ranged

31   from synergistic at low exposures (50 ppb) to antagonistic at exposures of 200 ppb of each gas

1 (Khan and Khan, 1994). Although various physiological measurements were made in these and

2 earlier studies, it has not been possible to determine any consistent mechanism or mechanisms

3 that might account for the conflicting results.

4   Since the information available about $O_3 \times SO_2$ interactions appears to be highly dependent

5 upon species, the type of response measured, and the experimental protocol used, it would still

6 appear prudent to heed the statement of Heagle et al. (1988) in their summary of the studies

7 undertaken in 12 field experiments over several years within the NCLAN program: "There were

8 no cases where O_3 and SO_2 interactions *significantly* affected yield." (emphasis added.)

9

10 **9.4.6.3   Nitrogen Oxides, Nitric Acid Vapor, and Ammonia**

11   The major oxides of nitrogen that occur in ambient air are nitrous oxide (N_2O), nitric oxide

12 (NO) and nitrogen dioxide (NO_2), of which the latter two (conveniently symbolized as NO_x) are

13 particularly important in connection with O_3, because they are components of the reaction mix

14 that leads to photochemical O_3 formation and because they can interact with O_3-responses. Their

15 reactions in the atmosphere can also lead to the occurrence of nitric acid vapor (HNO_3) in

16 ambient air. The other major N-containing contaminant of ambient air in many parts of the

17 world is ammonia (NH_3), largely released through agricultural practices.

18   Despite various combinations of O_3 and NO_x being probably the most common air

19 pollutant combinations found in the field, Barnes and Wellburn (1998) noted that they have been

20 little studied. Much early work with O_3 and NO_x focused on $O_3 \times NO_2$ interactions and can be

21 discounted, because of the unrealistic concentrations employed and their use as mixtures rather

22 than in types of sequences. The 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996)

23 concluded that evidence from studies involving concurrent exposures to both O_3 and NO_x at

24 realistic concentrations was so fragmented and varied that no firm conclusions could be drawn as

25 to the likelihood and nature such interactions. However, the few recent investigations taken

26 together with the earlier data are now beginning to reveal a pattern of response.

27   With regard to NO, Nussbaum et al. (1995a; 2000b) reported their findings with concurrent

28 exposures to NO and O_3 and observed that, at low O_3 levels, NO tended to act similarly to O_3 by

29 increasing the scale of responses such as growth reductions. However, in ambient air in which

30 O_3 is a dominant factor, the effects of NO were usually found to be negligible due to low levels,

1   although the authors admitted that the effects observed were confounded by the inevitable

2   O_3-induced oxidation of NO to NO_2.

3   Two possible mechanisms whereby NO may influence plant response to O_3 are suggested

4   by recent biochemical studies. First, there is growing evidence for the role of NO as a signaling

5   agent in plants that can induce defense responses to a range of biotic and abiotic stressors

6   (Beligni and Lamattina, 2001; Neill et al., 2002). Second, a role for NO as an antioxidant

7   scavenger of reactive oxygen species has been demonstrated by Beligni and Lamattina (2002) in

8   potato (*Solanum tuberosum*) leaves and chloroplasts. However, both of these cases concern

9   endogenously synthesized NO, and it must be noted that in none of these or other reports of

10   studies of NO signaling have the authors considered the potential significance of exogenous NO

11   in ambient air.

12   An independent case for $O_3 \times$ NO interactions comes from Mills et al. (2000). The ANN

13   model developed to predict the O_3 effects on white clover (*Trifolium repens*) biomass based on

14   experiments at 18 locations throughout Europe found that the minimum daily NO concentration

15   (at 5 p.m.) was a significant contributor to adverse effects.

16   Turning to NO_2, Maggs and Ashmore (1998) found that, although concurrent but

17   intermittent exposures of Bismati rice (*Oryza sativa*) revealed no significant growth interactions,

18   NO_2 reduced the rate of O_3-induced senescence, an antagonistic response possibly related to

19   enhanced N-metabolism.

20   With regard to sequential exposures, two studies on gene activation in tobacco (*Nicotiana*

21   *tabacum*) revealed that NO_2 counteracted the effect of O_3 in reducing mRNA levels for three

22   genes encoding photosynthetic proteins (Bahl and Kahl, 1995) and tended to counteract the

23   O_3-induced enhancement of defense-protein gene activation (Bahl et al., 1995). However,

24   despite compelling evidence for significant interactive effects provided by earlier studies

25   (Bender et al., 1991; Goodyear and Ormrod, 1988; Runeckles and Palmer, 1987), the only recent

26   investigation of growth effects seems to have been that of Mazarura (1997) using sine-wave

27   exposure profiles. He found that although 4 weeks of twice daily 3-h exposures to NO_2 (120 ppb

28   peak concentrations) slightly stimulated growth of radish (*Raphanus sativa*) and while daily 6-h

29   exposures to O_3 (120 ppb peak concentration) did not significantly reduce growth, the daily

30   sequence, NO_2 - O_3 - NO_2, led to a 13% drop in dry matter production.

1   The combined evidence to date, therefore, suggests that, in leguminous species, the effects

2   of these sequences are antagonistic with NO_2 tending to reduce (or reverse) the negative effects

3   of O_3 on growth, while the effects are increased in other species. These conclusions differ from

4   those of Barnes and Wellburn (1998) who suggested that sequential exposures tended to result in

5   antagonistic effects (largely based on the summary by Bender and Weigel, 1992), whereas

6   simultaneous exposures were likely to lead to synergistic responses. With disagreements both

7   among the data and their interpretation, it is not possible to determine the circumstances under

8   which specific interactions of O_3 and NO_2 may occur, but there is no reason to doubt the validity

9   of the individual findings of each study. Far more systematic investigation is needed to clarify

10   the situation.

11   There appear to have been no studies of O_3 interacting with HNO_3 in the vapor phase.

12   However, in the southern California montane forests (Takemoto et al., 2001), in Sweden (Janson

13   and Granat, 1999), and elsewhere, significant amounts of N are deposited in this form because of

14   the vapor's high deposition velocity. As a consequence, although much of it ultimately reaches

15   the ground through leaching and leaf fall and enters the soil as nitrate (NO_3^-), it may also be used

16   as a N source by the foliage itself. This nutritional role is independent of any contribution that

17   HNO_3 vapor may make to acidic deposition. Indirect interactions with the effects of O_3 through

18   N-deposition of NO_x, HNO_3, and NH_3 are related to the interactions of O_3 with N as a nutrient,

19   and have recently been examined in the review by Takemoto et al. (2001). The 1996 O_3 AQCD

20   (U.S. Environmental Protection Agency, 1996) stated that the evidence available at that time led

21   to estimates of total forest dry deposition, including HNO_3, ranging from 5.7 to 19.1 kg N ha^{-1}

22   year^{-1} (Taylor, Jr. et al., 1988). However, Takemoto et al. (2001) pointed out that in parts of the

23   mid-elevation forests of southern California, dry deposition rates may reach more than 40 kg N

24   ha^{-1} year^{-1}. As a result, some locations have seen the conversion from N-limited to N-saturated

25   forests. The concern for California's forests is well-stated by Takemoto et al.: "As potential

26   modifiers of long-term forest health, O_3 is a stressor and N deposition is an enhancer of

27   ponderosa/Jeffrey pine physiology and growth (Grulke and Balduman, 1999). The progression

28   toward a deciduous growth habit, higher shoot:root biomass ratios, increasing depths of litter,

29   tree densification, and elevated NO_3^- levels in soil and soil solution, all point to the replacement

30   of pine species with nitrophilous, shade- and O_3-tolerant tree species, such as fir and cedar

31   (Minnich et al., 1995) (Minnich, 1999)."

1   Few studies have been reported of interactions of O_3 with NH_3. The 1996 criteria

2   document made reference to the work on bean (*Phaseolus vulgaris*) by Tonneijck and van Dijk

3   (1994, 1998). Although NH_3 alone tended to increase growth and O_3 alone to inhibit it, one

4   interaction was noted (Tonneijck and van Dijk, 1994) on the number of injured leaves. Dueck

5   et al. (1998) studied the effects of O_3 and NH_3 on the growth and drought resistance of Scots

6   pine (*Pinus sylvestris*). Significant interactions were found for some growth features, but there

7   were no consistent patterns of the effects of NH_3 on O_3 response or vice versa. However, O_3 was

8   found to ameliorate the enhancement of drought stress caused by NH_3.

9   At this time there is insufficient information to offer any general conclusions about the

10   interactive effects of O_3 and NH_3.

11

12 **9.4.6.4 Hydrogen Fluoride and Other Gaseous Pollutants**

13   Although HF and other fluorides are important local air pollutants associated with

14   aluminum smelting and superphosphate fertilizer manufacture, no studies of possible interactions

15   with oxidants appear to have been reported since that of MacLean (1990). He found that HF

16   retarded the accelerated senescence and loss of chlorophyll resulting from O_3 exposure in corn

17   seedlings. However, such an isolated observation cannot be taken to indicate that HF can reduce

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

19   mature plants.

20

21 **9.4.6.5 Acid Deposition**

22   The deposition of acidic species onto vegetation may elicit direct effects on the foliage or

23   indirect effects via changes induced in the soil. The 1996 O_3 AQCD (U.S. Environmental

24   Protection Agency, 1996) included an extensive listing of investigations into the effects of O_3

25   and acid deposition (usually in the form of simulated acid rain, SAR) on plant growth and

26   physiology. The majority of studies found no effects of SAR or acid mists or fogs at pH values

27   greater than about 3.0 and no interactive effects with O_3. (In ambient air, pH values less than 3.0

28   have rarely been reported.) In the few reports in which significant interactions were found, most

29   were antagonistic and were explained as probably being the result of increased fertility due to

30   nitrate and sulfate supplied in the SAR.

1   Although numerous reviews have recently appeared (e.g., Bussotti and Ferretti, 1998;

2   Flückiger et al., 2002; Fox and Mickler, 1996; Nussbaum et al., 1999; and Sheppard and Cape,

3   1999), the shift in interest in air pollution effects away from acid deposition has resulted in little

4   new research having been reported over the past 10 or so years. In most of the reported studies,

5   no effects due to the O_3 exposures, the SAR treatments used, or their combinations were

6   observed, e.g., Baker et al. (1994) on loblolly pine (*Pinus taeda*); Laurence et al. (1997), and

7   Vann et al. (1995) on red spruce (*Picea rubens*); and Laurence et al. (1996) on sugar maple

8   (*Acer saccharum*). Branch chamber studies of 12-year old Ponderosa pine (*Pinus ponderosa*)

9   trees by Momen et al. (1997, 1999) revealed no O_3 effects or interactions. With red spruce

10   (*Picea rubens*), Sayre and Fahey (1999) noted no effects of O_3 on the foliar leaching of Ca or

11   Mg, which only became significant with SAR at pH 3.1. Izuta (1998) observed no interactions

12   with Nikko fir (*Abies homolepis*), although SAR at ph 4.0 reduced dry matter. Shan et al. (1996)

13   reported adverse effects of O_3 but none attributable to SAR on the growth of *Pinus armandi*.

14   With herbaceous species, Ashenden et al. (1995) noted significant antagonistic interactions

15   of O_3 and acid mist in white clover (*Trifolium repens*), in which the adverse effect of low pH was

16   countered by O_3. In contrast, Ashenden et al. (1996) found that, although pH 2.5 mist caused a

17   significant stimulation of the growth of ryegrass (*Lolium perenne*) attributed to a fertilizer effect,

18   and O_3 caused reduced growth, there was no interaction. Bentgrass (*Agrostis capillaris*) behaved

19   similarly.

20   A study by Bosley et al. (1998) on the germination of spores of the moss, *Polytrichum*

21   *commune*, and the ferns, *Athyrium felix-femina* and *Onoclea sensibilis*, revealed no effect of O_3

22   on moss spores, while SAR at pH < 4.0 was completely inhibitory. With the ferns, germination

23   was progressively reduced by both increased O_3 and acidity.

24   In summary, the few findings of interactions in these recent studies are consistent with the

25   previous conclusion regarding the likelihood of such interactions being antagonistic. However,

26   the interactions observed were in each case largely the result of the response to the lowest pH

27   used, which in several cases was below 3.0, and hence may not be relevant to most field

28   conditions.

29

30

1 **9.4.6.6 Heavy Metals**

2 Since there appears to have been no further research into the interactions of oxidants with

3 heavy metal pollutants, our understanding is unchanged from at the time of the 1996 O_3 AQCD

4 (U.S. Environmental Protection Agency, 1996). As noted therein, the limited data available

5 from early studies indicates varying degrees of enhancement of any adverse effects of O_3 but

6 precludes the development of any response relationships.

7

8 **9.4.6.7 Mixtures of Ozone with Two or More Pollutants**

9 In many airsheds the mixtures that occur, both concurrently and over time, may involve

10 three or more pollutants. Very little useful information exists on the effects of O_3 with multiple

11 pollutants. As the 1996 criteria document and others have pointed out, most of the early studies

12 on such combinations can be discounted because of their use of (1) high and environmentally

13 irrelevant exposure concentrations and (2) unrealistic, repetitive exposure profiles (U.S.

14 Environmental Protection Agency, 1996; Barnes and Wellburn, 1998).

15 The large investment in experimental facilities required to study these complex interactions

16 is a major deterrent. So, although the topic has been included in several reviews that have

17 appeared in the last decade, there appear to have been only two studies that have provided new

18 information on the effects of O_3 in combination with more than one other pollutant stress.

19 Ashenden et al. (1995, 1996) studied the effects of O_3 and/or (SO_2 + NO_2) with four acidities of

20 SAR applied to each gas treatment, on white clover (*Trifolium repens*) and two pasture grasses

21 (*Lolium perenne* and *Agrostis capillaris*). With each species, the antagonism reported for the O_3

22 × SAR interaction (Section 9.4.4.6.5) tended to be nullified by concurrent exposure to the other

23 gases, while the combination of the three gaseous pollutants resulted in the most severe growth

24 inhibition, regardless of the acidity of the SAR.

25 With such meager evidence, no clear conclusions can be drawn as to the ways in which the

26 effects of multiple airborne stressors could influence or be influenced by O_3.

27

28 **9.4.7 Interactions with Agricultural Chemicals**

29 The review of interactions involving O_3, plants, and various agricultural chemicals

30 presented in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) remains a valid

31 assessment of our limited knowledge of these interrelationships. Our knowledge is largely based

1   on the protection against O_3 afforded to a range of crop species by applications of various

2   chemicals, particularly fungicides, such as benomyl (benlate; methyl-1-[butylcarbamoyl]-2-

3   benzimidazolecarbamate) and several carbamates and triazoles. A recent report has added

4   azoxystrobin (AZO) and epoxyconazole (EPO) to the list (Wu and Tiedemann, 2002). Foliar

5   sprays of either AZO or EPO provided 50 to 60% protection against O_3 injury to barley

6   (*Hordeum vulgare*) leaves. Both had similar modes of action involving stimulation of the levels

7   of antioxidant enzymes such as SOD, ascorbate peroxidase, guaiacol peroxidase, and catalase.

8   In contrast, applications of herbicides have yielded variable results ranging from increased

9   sensitivity to protection from O_3; the nature of the effect is usually species- or cultivar-

10   dependent. Although of less wide application, some plant growth retardants have also been

11   found to provide protection, but no insecticide appears to have been clearly shown to have

12   similar properties.

13   Despite the attraction of the use of permitted chemicals to provide crop protection, the

14   statement in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) is still valid:

15   "It is premature to recommend their use specifically for protecting crops from the adverse effects

16   of O_3, rather than for their primary purpose."

17

18   ## 9.4.8   Factors Associated with Global Climate Change

19   During the last decade, interest in the effects of climatic change on vegetation has replaced

20   concerns over the purported causes of forest decline and the effects of acidic deposition. Two

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

22   activity:

23   • the effects of increasing mean global CO_2 concentrations in the lower atmosphere, and

24   • the effects of increasing levels of surface-level irradiation by UV-B (the result of
        stratospheric O_3 depletion).

25   In spite of the crucial role of temperature as a climatic determinant (Monteith and Elston, 1993),

26   the effects of increasing mean global temperatures and their interactions with increasing CO_2

27   levels in particular have received less attention.

28   All of the biotic and chemical interactions with oxidants discussed in the preceding

29   sections may be modified by these climatic changes. However, research activities have largely

1   focused on the two-way $O_3 \times CO_2$ interaction. Little if any experimental evidence exists related

2   to three-way interactions such as $O_3 \times CO_2 \times$ disease or $O_3 \times CO_2 \times$ nutrient availability,

3   although such interactions cannot be predicted from the component two-way interactions.

4   Numerous reviews have appeared since the 1996 O_3 AQCD (U.S. Environmental

5   Protection Agency, 1996) dealing with the issues involved. General reviews include

6   publications of IPCC (1996); IPCC (2001); and UNEP (1993, 1999); the volume by Wellburn

7   (1994); the volumes edited by Alscher and Wellburn (1994), De Kok and Stulen (1998), Singh

8   (2000), and Yunus and Iqbal (1996); and papers by Idso and Idso (1994), Krupa and Groth

9   (2000), Luo et al. (1999), Polle and Pell (1999), Poorter and Pérez-Soba (2001), Runeckles

10   (2002), and Weubbles et al. (1999). Effects on agriculture and crop production, growth, and

11   metabolism have been reviewed by Groth and Krupa (2000), Rötter et al. (1999), and Schnug

12   (1998); effects on forests have been reviewed by Bortier et al. (2000); with focus on insect pests,

13   Docherty et al. (1997), Karnosky et al. (2001b,a), McLaughlin and Percy (1999), and Saxe et al.

14   (1998).

15   As background to the discussion of interactions with O_3, it should be noted that the

16   increased levels of CO_2 experienced since the mid-18th century are such that, without abatement

17   of the rates of increase, increased levels of from 540 to 970 ppm have been projected by the year

18   2100 (IPCC, 2001). Such increases in the concentration of CO_2, the principal GHG released into

19   the atmosphere, will inevitably lead to increased global mean temperatures, evidence for which

20   is already available from oceanic, icepack, and other records. The latest estimates of the global

21   warming are for an increase in the range 1.4 to 5.8 °C over this century, in contrast to the 0.6 °C

22   rise experienced since 1900 (IPCC, 2001). However, considerable uncertainty is associated with

23   such projections of future increases in global temperature.

24   The use of elevated CO_2 concentrations has been common practice for many years in the

25   production of many greenhouse crops. Much of our early knowledge of the effects of higher

26   than ambient CO_2 levels on plant growth derives from this application, coupled with research of

27   plant physiologists on how CO_2 concentrations affect the process of photosynthesis. Information

28   available about effects of increased CO_2 levels on photosynthesis and stomatal function, in

29   particular, has provided the underlying bases for numerous process models that simulate plant

30   growth under stress and in changed climates.

1   Although simple $O_3 \times$ temperature interactions were discussed in Section 9.4.4.2, the close

2   linkage between global CO_2 levels and global mean temperatures in the context of climate

3   change requires that an assessment of the interactive effects with O_3 should focus, as much as

4   possible, on interactions involving all three factors.

5

6   **9.4.8.1   Ozone-Carbon Dioxide-Temperature Interactions**

7   Idso and Idso (1994) reviewed several hundred reports published between 1982 and 1994

8   on the effects of increased CO_2 on plant growth and net photosynthesis. Their survey covered a

9   wide range of temperate and tropical, herbaceous and perennial species, including coniferous

10   trees. They concluded that, for responses to a 300-ppm increase in CO_2, somewhat less than a

11   doubling of present-day levels, but somewhat greater than the 540 ppm lower limit suggested by

12   the IPCC (2001), averaged across all species:

13   - light intensity had a negligible effect on net photosynthesis other than at limiting low
     intensities under which the CO_2-driven enhancement was increased;

14   - increased temperature tended to increase the CO_2-driven enhancement of dry matter
     accumulation (growth) and net photosynthesis;

15   - drought conditions tended to increase the CO_2-driven enhancements of both growth and
     net photosynthesis, but increased salinity had little effect;

16   - mineral nutrient deficiency (especially of nitrogen) tended to increase the CO_2-driven
     enhancement of growth; and

17   - in the presence of air pollutants (especially SO_2 and NO_x), the CO_2-driven enhancement
     of net photosynthesis tended to be increased.

18   It should be noted that the statement that CO_2-enhanced growth increased with temperature

19   referred to total dry matter accumulation by the whole plant and not to the yield of grain, fruit, or

20   seed. Unfortunately, despite the existence of several reports at the time, the summary of

21   interactions with air pollutants contained only a single reference to O_3, i.e., Pfirrmann and

22   Barnes (1993), who reported surprisingly that a doubling of CO_2 levels led to a 27% increase in

23   dry weight of radish (*Raphanus sativus*) but that the combination with O_3 led to a 77% increase.

24   The more recent reviews by Rudorff et al. (2000) and Olszyk et al. (2000) have addressed

25   $CO_2 \times O_3$ interactions in detail, with the latter focusing on the implications for ecosystems. They

26   concluded that:

1   • the effects of both gases on stomatal closure were predominantly additive, with little evidence of interaction;

2   • increased photosynthesis resulting from elevated CO_2 may be canceled by exposures to high O_3 levels;

3   • foliar O_3 injury is reduced by elevated CO_2; and

4   • interactions between CO_2 and O_3 can affect storage carbohydrates, leaf free-radical metabolism, and carbon allocation to shoots and roots.

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

6   Much of the recently published information on the effects of increased CO_2 and O_3 levels is

7   summarized in Table 9-12. Note that the table only lists the *directions* of O_3-induced effects and

8   any modifications of these effects resulting from elevated CO_2, not their magnitudes. These

9   directions are usually, but not necessarily, the same as the corollary effects of O_3 on

10   CO_2-induced responses.

11   The bulk of the available evidence clearly shows that, under the various experimental

12   conditions used (which almost exclusively employed abrupt or "step" increases in CO_2

13   concentration, as discussed below), increased CO_2 levels may protect plants from the adverse

14   effects of O_3 on growth. This protection may be afforded in part by CO_2 acting together with O_3

15   in inducing stomatal closure, thereby reducing O_3 uptake, and in part by CO_2 reducing the

16   negative effects of O_3 on Rubisco and its activity in CO_2-fixation. However, the situation with

17   regard to the combined effects of O_3 and CO_2 on stomatal functioning is not clear-cut. In spite of

18   the wealth of evidence supporting both CO_2-induced and O_3-induced short-term stomatal closure,

19   studies over long-term exposures, especially with tree species, have revealed little effect of

20   elevated CO_2 on stomatal conductance. Reverse effects of O_3 on stomata have also been noted,

21   as a result of O_3-induced stomatal dysfunction after extended periods of exposure (Maier-

22   Maercker, 1998).

23   At the mechanistic level, Rubisco plays a key role in CO_2-assimilation, and while both O_3

24   and elevated CO_2 per se can lead to reduced activity, CO_2 can also reverse the O_3-induced

25   inhibition of Rubisco activity and photosynthesis (Table 9-12). However, in their review of the

26   possible mechanisms involved, Polle and Pell (1999) cautioned that Rubisco should not be

27   regarded "as a unique target for the interaction of the two gases." But it is clear from the bulk of

28

Table 9-12. 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 (*Triticum aestivum*)	CSTR, P	Rao et al. (1995)
	∨	○	Sugar maple (*Acer saccharum*)	CEC, P	Niewiadomska et al. (1999)
	∨	○	Trembling aspen (*Populus tremuloides*)	FACE, G	Wustman et al. (2001)
Catalase	∨	○	Wheat (*T. aestivum*)	CEC, P	McKee et al. (1997b)
	∧	○		CEC, P	Niewiadomska et al. (1999)
Chlorophyll	∨	▼	Wheat (*T. aestivum*)	OTC, G	Ommen et al. (1999); Donnelly et al. (2000)
	∨	▼	Potato (*Solanum tuberosum*)	OTC, G	Donnelly et al. (2001a)
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)
Rubisco	∨	▼	Soybean (*Glycine 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. (2001)
	∨	▼	Sugar maple (*A. saccharum*)	CEC, P	Gaucher et al. (2003)

Table 9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

O₃ Effects: ∨, Decrease; ∧, Increase; ◯, No Significant Effect.
CO₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ◯, No Significant Effect.

Plant response	O₃ Response[a]	CO₂ 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 (*Fagus 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 (*Raphanus sativus*)	CEC, P	Barnes and Pfirrmann (1992)
	∨	▼	Soybean (*G. max*)	OTC, G	Mulchi et al. (1992)
	∨	▼	Bean (*Phaseolus vulgaris*)	OTC, P	Heagle et al. (2002)
	∨	[▲]	White clover (*Trifolium repens*) (O₃-sensitive)	CSTR, P	Heagle et al. (1993)
	∧ - ◯▯	▼ - ◯▯	White clover (*T. repens*) (O₃-tolerant)	CSTR, P	Heagle et al. (1993)
	∨	▲	Tomato (*Lycopersicon esculentum*)	CEC, P	Hao et al. (2000)

Table 9-12 (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; O, No Significant Effect.				
	CO_2 Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; O, 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)
	∨	O□		OTC, G	Mulholland et al. (1997b)
	∨	O - ▲		CEC, P	Donnelly et al. (1998)
	∧	O□		CEC, P	Tiedemann and Firsching (2000)
	∨	O□	*Agropyron smithii*	CEC, P	Volin et al. (1998)
	∨	O□	*Koeleria cristata*	CEC, P	Volin et al. (1998)
	O□	O□	*Bouteloua curtipendula*	CEC, P	Volin et al. (1998)
	O□	O□	*Schizachyrium scoparium*	CEC, P	Volin et al. (1998)
	O□	O□	Black cherry (*Prunus serotina*)	CSTR, P	Loats and Rebbeck (1999)
	O□	O□	Green ash (*Fraxinus pennsylvanica*)	CSTR, P	Loats and Rebbeck (1999)
	[∧]	▼	Yellow poplar (*Liriodendron tulipifera*)	CSTR, P	Loats and Rebbeck (1999)
	∨	▲	Trembling aspen (*P. tremuloides*)	CEC, P	Volin et al. (1998)

Table 9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

	O₃ Effects: ∨, Decrease; ∧, Increase; O, No Significant Effect.				
	CO₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; O, No Significant Effect.				
Plant response	**O₃ Response[a]**	**CO₂ Codification[b]**	**Species**	**Facility[c]**	**Reference**
Physiological (cont'd)					
Stomatal conductance (cont'd)	O - ∨	▼		FACE, G	Noormets et al. (2001)
	O■	O■	Red oak (*Quercus rubra*)	CEC, P	Volin et al. (1998)
	[∧]	▼	Durmast oak (*Quercus 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	Mulchi et al. (1992)
	∨	▼	Bean (*P. vulgaris*)	OTC, P	Heagle et al. (2002)
	∨	O■	Tomato (*L. esculentum*)	CEC, P	Hao et al. (2000)
	O■	O■	Potato (*S. tuberosum*)	OTC, G	Donnelly et al. (2001a)
	O■	[▼]		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)
				OTC, P	Reid and Fiscus (1998)
				CEC, P	Tiedemann and Firsching (2000)
	∨	O■		CEC, P	Cardoso-Vilhena and Barnes (2001)

Table 9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

	O₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect.				
	Co₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.				
Plant response	**O₃ Response[a]**	**CO₂ Codification[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 (*Pinus ponderosa*)	CEC, G	Olszyk et al. (2001)
	∨	▼	Scots pine (*Pinus sylvestris*)	OTC, G	Kellomäki and Wang (1997b); Kellomäki and Wang (1997a)
	○◻	○◻	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 9-12 (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$_2$ Codification[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 (*Petroselinum 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	Mulchi et al. (1992) Reinert et al. (1997)
	[⋁]	○▯	Alfalfa (*Medicago 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 9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

	O₃ Effects: ∨, Decrease; ∧, Increase; ◯, No Significant Effect. CO₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ◯, No Significant Effect.				
Plant response	**O₃ Response[a]**	**CO₂ Codification[b]**	**Species**	**Facility[c]**	**Reference**
Growth, Yield (cont'd)					
Total biomass (cont'd)	∨	◯		OTC, G	Lawson et al. (2001a)
	∨	▼	Mustard (*Sinapis alba*)	CEC, P	Cardoso-Vilhena et al. (1998)
	∨	▼	Plantain (*Plantago major*)	CEC, P	Cardoso-Vilhena et al. (1998)
	∨	▼	Cotton (*Gossypium hirsutum*)	OTC, P	Booker, 2000) Heagle et al. (1999)
	∨	▼	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. (2000) 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 (*Phleum pratense*)	CEC, P	Johnson et al. (1996a)
	∨	▼	*Agropyron smithii*	CEC, P	Volin et al. (1998)
	∨	▼	*Koeleria cristata*	CEC, P	Volin et al. (1998)

Table 9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

	O$_3$ Effects: $\vee$, Decrease; $\wedge$, Increase; $\bigcirc$, No Significant Effect.				
	Co$_2$ Effects: $\blacktriangle$, Additive/Synergistic; $\blacktriangledown$, Antagonistic/Ameliorative; $\bigcirc$, No Significant Effect.				
Plant response	**O$_3$ Response[a]**	**CO$_2$ Codification[b]**	**Species**	**Facility[c]**	**Reference**
Growth, Yield (cont'd)					
Total Biomass (cont'd)	$\vee$	$\blacktriangledown$	Corn (*Zea mays*)	OTC, G	Rudorff et al. (1996a)
	$\vee$	$\bigcirc\square$	*Bouteloua curtipendula*	CEC, P	Volin et al. (1998)
	$\bigcirc$	$\bigcirc\square$	*Schizachyrium scoparium*	CEC, P	Volin et al. (1998)
	$\vee$	$\blacktriangledown$	Ponderosa pine (*P. ponderosa*)	CEC, G	Olszyk et al. (2001)
	$\vee$	$\blacktriangle$	Birch (*Betula pendula*)	CEC, P	Kytöviita et al. (1999)
	[$\vee$]	$\bigcirc\square$	Black cherry *P. serotina*	CSTR, P	Loats and Rebbeck (1999)
	$\wedge$	$\blacktriangledown$	Green ash (*F. pennsylvanica*)	CSTR, P	Loats and Rebbeck (1999)
	$\bigcirc$	$\bigcirc\square$	European ash (*Fraxinus excelsior*)	OTC, G	Broadmeadow and Jackson (2000)
	[$\wedge$]	$\blacktriangle$	Yellow poplar (*L. tulipifera*)	CSTR, P	Loats and Rebbeck (1999)
	$\vee$	$\blacktriangledown$	Sugar maple (*A. saccharum*)	CEC, P	Gaucher et al. (2003)
	$\vee$	$\blacktriangledown$	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)
	$\vee$	$\bigcirc\square$	(O$_3$-sensitive clone)	OTC, G	Dickson et al. (2001)
	$\bigcirc$	$\bigcirc\square$	Red oak (*Q. rubra*)	CEC, P	Volin et al. (1998)
	$\vee$	$\blacktriangledown$	Durmast oak (*Q. petraea*)	CEC, P OTC, G	Broadmeadow et al. (1999) Broadmeadow and Jackson (2000)

Table 9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

	O₃ Effects: ∨, Decrease; ∧, Increase; ○, No Significant Effect. Co₂ Effects: ▲, Additive/Synergistic; ▼, Antagonistic/Ameliorative; ○, No Significant Effect.				
Plant response	**O₃ Response[a]**	**CO₂ Codification[b]**	**Species**	**Facility[c]**	**Reference**
Growth, Yield (cont'd)					
Total Biomass (cont'd)	○	○■	Aleppo pine (*Pinus 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	Fiscus et al. (1997); Mulchi et al. (1992); Mulchi et al. (1995)
	∨	▼	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. (1998a) Rudorff et al. (1996b)
	[∨]	▼	Wheat (*T. aestivum*) (cont.)	OTC, G	Fangmeier et al. (1996)
	∨	[▼]		OTC, G	Mulholland et al. (1998b, 1998a)

Table 9-12 (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$_2$ Codification[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)
	∨	○▯		OTC, G	Mulchi et al. (1992)

Table 9-12 (cont'd). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

O$_3$ Effects: $\vee$, Decrease; $\wedge$, Increase; O, No Significant Effect.
Co$_2$ Effects: $\blacktriangle$, Additive/Synergistic; $\blacktriangledown$, Antagonistic/Ameliorative; O, No Significant Effect.

Plant response	O$_3$ Response[a]	CO$_2$ Codification[b]	Species	Facility[c]	Reference
Growth, Yield (cont'd)					
Specific leaf area-SLA (cont'd)	$\vee$	$\blacktriangle$	White clover (*T. repens*) (O$_3$-sensitive)	CSTR, P	Heagle et al. (1993)
	$\wedge$	$\blacktriangle$	White clover (*T. repens*) (O$_3$-tolerant)	CSTR, P	Heagle et al. (1993)
	$\wedge$	$\blacktriangle$	*Agropyron smithii*	CEC, P	Volin et al. (1998)
	$\wedge$	$\blacktriangle$	*Koeleria cristata*	CEC, P	Volin et al. (1998)
	O	O	*Bouteloua curtipendula*	CEC, P	Volin et al. (1998)
	$\wedge$	O	*Schizachyrium scoparium*	CEC, P	Volin et al. (1998)
	$\wedge$	$\blacktriangle$	Trembling aspen (*P. tremuloides*)	CEC, P	Volin et al. (1998)
	$\wedge$	$\blacktriangle$	Red oak (*Q. rubra*)	CEC, P	Volin et al. (1998)
Root/shoot ratio	$\vee$	$\blacktriangledown$	Radish (*R. sativus*)	CEC, P	Barnes and Pfirrmann (1992)
	$\vee$	$\blacktriangle$	Alfalfa (*M. sativa*)	CEC, P	Johnson et al. (1996a)
	$\vee$	$\blacktriangledown$	White clover (*T. repens*) (O$_3$-sensitive)	CSTR, P	Heagle et al. (1993)
	O	O▯	White clover (*T. repens*) (O$_3$-tolerant)	CSTR, P	Heagle et al. (1993)
	$\wedge$	$\blacktriangle$	Wheat (*T. aestivum*)	CEC, P	McKee et al. (1997a)

Table 9-12 (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$_2$ Codification[b]	Species	Facility[c]	Reference
Growth, Yield (cont'd)					
Root/shoot ratio (cont'd)	∨	▼	Timothy (*P. pratense*)	CEC, P	Johnson et al. (1996a)
	○	○❲▼❳	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)
	∧	▼	Cotton (*G. hirsutum*)	OTC, P	Heagle et al. (1999)
	∧	▼	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*.

1   the evidence in Table 9-12 that elevated CO_2 levels can ameliorate the inhibition of growth

2   caused by O_3 in many species, although the precise balance among the mechanisms involved

3   may well vary from species to species. Three important caveats must be raised with regard to

4   the findings presented in Table 9-12:

- 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 stresses 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 (IPCC, 2001).

8   The first caveat concerns the distinctly different natures of the exposures to O_3 and CO_2

9   experienced by plants in the field. Changes in the ambient concentrations of these gases have

10   very different dynamics. In the context of climate change, CO_2 levels increase relatively slowly

11   and may change little over several seasons of growth. On the other hand, O_3 presents a

12   fluctuating stress with considerable hour-to-hour and day-to-day variability (Polle and Pell,

13   1999). Almost all of the evidence presented in Table 9-12 comes from experimentation

14   involving plants grown from the outset in, or subjected to, an abrupt or step increase to a higher

15   (more or less double), steady CO_2 concentration. In contrast, the O_3 exposure concentrations

16   usually varied from day to day. Luo and Reynolds (1999), Hui et al. (2002), and Luo (2001)

17   noted the difficulties in predicting the likely effects of a gradual CO_2 increase from experiments

18   involving a step increase or those using a range of CO_2 concentrations. Indeed, although using

19   the much accelerated time-scale of an 80-day growing season, Hui et al. (2002) clearly showed

20   significant differences between the rates and magnitudes of various physiological and growth

21   responses of plantain (*Plantago lanceolata*) to CO_2 between gradual and step increase

22   treatments. The authors concluded that, even though there were major differences in most of the

23   parameters studied between the gradual and step treatments, "the convergence of the measured

24   parameters at the end of the experiment provides some encouragement for the applicability of

1 step-type experiments in the field; however, the study suggests caution in interpreting early

2 results from short-term studies."

3 　　In long-term studies, the matter of photosynthetic acclimation to elevated CO_2 levels has to

4 be considered. Lawlor and Keys (1993) define *acclimation* in terms of long-term (days, weeks),

5 irreversible physiological changes, in contrast to *regulation*, which relates to more rapid

6 (minutes, hours), reversible changes. Each may be positive or negative, but many studies

7 indicate that, while positive acclimation to elevated CO_2 levels initially led to enhanced

8 photosynthesis and growth, negative acclimation ultimately ensued and reduced CO_2

9 assimilation and growth rates. However, the consensus from recent studies and reviews is that

10 such negative acclimation is most likely to occur in situations in which plants are grown under

11 some additional stress, induced, e.g., by limitations to growth posed by lack of resources such as

12 water or nutrients. The meta-analysis by Curtis (1996) revealed that slow or little negative

13 acclimation was noted in studies on unstressed tree species with unhindered opportunities for

14 root growth and development, a view originally suggested by Arp and Drake (1991) and largely

15 supported in the review by Eamus (1996). A nonwoody perennial, the rhizomatous wetland

16 sedge, *Scirpus olneyi,* grown in its natural environment with no edaphic limitations showed no

17 negative acclimation after 4 years; in fact, photosynthetic capacity increased by 31% (Arp and

18 Drake, 1991). No negative acclimation of well-watered, field-grown Ponderosa pine (*Pinus*

19 *ponderosa*) trees was observed by Tissue et al. (1999) after 6 years of growth at 2×-ambient CO_2

20 levels. Gifford and Morison (1993) have summarized the situation thus: "Where the aerial or

21 root environment for a plant is restricted (as with inter-plant competition, for example), positive

22 feedback is limited and adjustments to the changed resource input balance under high CO_2 can

23 include 'down-regulation' of leaf photosynthesis rate as an integral part of a positive growth

24 response."

25 　　The influence of other environmental stress factors is borne out by several long-term tree

26 studies. After 3 years in 565 ppm CO_2 in the Duke Forest free-air CO_2 enrichment (FACE)

27 facility in North Carolina, maturing loblolly pine (*Pinus taeda*) trees showed only a marginal

28 CO_2-induced carbon gain if grown on a nutritionally moderate site, but zero gain if grown on a

29 nutritionally poor site (Oren et al., 2001). This is in sharp contrast to the substantially increased

30 initial growth rates in elevated CO_2 reported by DeLucia et al. (1999), but it is supported by the

31 observations of Tognetti et al. (2000) on five Mediterranean tree species growing for many years

1   adjacent to geothermal springs releasing CO_2 to provide ambient levels averaging 700 ppm.

2   No significant differences in radial growth of the oaks (*Quercus cerris*, *Q. ilex*, and

3   *Q. pubescens*), strawberry tree (*Arbutus Unedo*), and flowering ash (*Fraxinus Ornus*) could be

4   detected between trees at the naturally enriched site and those at a nearby site exposed to normal

5   ambient CO_2 (~350 ppm). The authors concluded that limited availability of water and nutrients

6   may have counteracted any positive effects of CO_2 on growth at the enriched site or that the trees

7   had acclimated to the higher CO_2 levels.

8   Because the ameliorative effects of CO_2 on responses to O_3 (Table 9-14) were reported

9   mostly in short-term studies involving an abrupt increase in CO_2 level, it is appropriate to ask

10   whether this amelioration is likely to persist to a time when the ambient CO_2 concentration is

11   relatively stable at such levels. Regardless of any negative acclimation due to resource

12   limitations that may occur in the interim, steadily rising CO_2 levels may well lead to natural

13   selection and genetic change. Nevertheless, it seems reasonable to expect that the amelioration

14   of O_3 impact at elevated CO_2 levels will be maintained in many situations, but the negative

15   acclimation that will probably occur in situations where other resources become limiting will

16   reduce the degree of protection.

17   Another caveat regarding the validity of some of the observations in Table 9-12 is related

18   to the matter of stress-induced negative acclimation to elevated CO_2 and concerns results

19   obtained using potted plants. Although much of the recent information on CO_2 effects has come

20   from experiments with plants rooted in the ground, more than half of the studies listed in

21   Table 9-12 used potted plants, whether in controlled environment and greenhouse chambers or in

22   OTCs. The recent meta-analysis of data on the effects of elevated CO_2 on soybean (*Glycine*

23   *max*) physiology and growth by Ainsworth et al. (2002) revealed a threefold smaller stimulation

24   of seed yield in pot-grown than in field-grown plants, even when large (> 9 L) pots were used.

25   Loats and Rebbeck (1999) noted that their inability to observe any CO_2-induced increases in the

26   root/shoot ratios in seedlings of three broad-leaved tree species (Table 9-13) may have resulted

27   from their use of potted plants. The use of potted plants was a confounding factor in the studies

28   of Taylor et al. (2001) of the differences in leaf growth of poplar (*Populus*) hybrids between

29   plants exposed to elevated CO_2 in controlled environment chambers (potted plants), OTCs or a

30   FACE facility. Eamus (1996) has suggested that any long-term observations of reduced stomatal

31   conductance are almost universally a consequence of pot-based trials at elevated CO_2 with

1   restricted root growth, in contrast to the lack of such a decline observed with trees rooted in the

2   ground. Although the majority of the cases cited in Table 9-12 indicate that O_3 and CO_2 act

3   additively or synergistically in causing stomatal closure, there are numerous exceptions.

4   Any reduction in stomatal aperture has consequences other than merely restricting O_3

5   uptake and the exchange of other gases. In particular, the rate of transpiration is reduced and,

6   while this tends to increase water-use efficiency, it may also lead to decreased mineral uptake,

7   which could adversely impact growth over extended periods. Furthermore, less transpiration

8   also means less evaporative cooling and an increase in leaf temperature, independent of any

9   change in mean air temperatures.

10   Hence, the final caveat regarding Table 9-12 concerns the interactions of O_3 and CO_2 with

11 other climatic variables, especially mean temperature. In light of the key role played by

12 temperature in regulating physiological processes and modifying plant response to increased

13 CO_2 levels (Long, 1991; Morison and Lawlor, 1999) and the knowledge that relatively modest

14 increases in temperature may lead to dramatic consequences in terms of plant development

15 (Lawlor, 1998), it is unfortunate that much of the large investment in time and resources spent

16 on recent studies of the effects of climate change on vegetation have gone into investigations

17 limited to increasing our knowledge of the effects of higher levels of CO_2 *at current ambient*

18 *temperatures*.

19   Some attention is now being paid to investigating the concurrent effects of CO_2 increases

20 and warming (recently reviewed by Rowland-Bamford, 2000 and Morison and Lawlor, 1999),

21 but the observed interactive effects on plant growth are inconsistent. For example, a FACE

22 study with ryegrass (*Lolium perenne*) ahowed that increased temperatures (provided by infrared

23 heaters) reduced the dry matter gain resulting from increased CO_2 levels (Nijs et al., 1996). The

24 field studies by Shaw et al. (2002) on a California annual grassland dominated by the grasses

25 *Avena barbata* and *Bromus hordeaceus* and the forbs *Geranium dissectum* and *Erodium botrys*

26 involved free-air increased CO_2 as well as increased temperature, precipitation, and N supply.

27 Not only did increased temperature reduce CO_2-stimulated net primary productivity (NPP), but

28 increased CO_2 itself, combined with other factors, was found to be able to cause reduced NPP.

29   There have been several investigations of effects on wheat (*Triticum aestivum*). Batts et al.

30 (1997) used plastic tunnels to create temperature gradients and maintain elevated CO_2 levels

31 over field-grown wheat and found that, in each of 4 years of study, a temperature rise of ~1.5 °C

1 consistently canceled the growth and yield increases caused by a doubling of the CO_2 level

2 above ambient. Similar findings were reported by van Oijen et al. (1999) and van Oijen and

3 Ewart (1999) in OTC field studies. Half of the chambers were cooled 1.6 to 2.4 °C below the

4 uncooled chambers, to cancel out the normal temperature increase over ambient, due to the

5 so-called "chamber effect" (usually a 1 to 3 °C increase above ambient temperature (Heagle

6 et al., 1988). Although temperature had no effect on CO_2-enhanced assimilation rates, the

7 CO_2-enhanced growth and grain yields observed in the cooled chambers were effectively

8 canceled out in the warmer chambers. The authors attributed this effect to accelerated

9 phenology, a shorter period for grain filling, and a lower leaf area index (LAI; total leaf area per

10 unit ground area) in the warmer chambers. Wheeler et al. (1996) observed that the benefit to

11 wheat of doubling the CO_2 level was offset by a mean seasonal increase of only 1 to 1.8 °C.

12 With the continuing use of OTCs for field research, Runeckles (2002), has suggested that the

13 temperature rise due to the chamber effect in OTCs should be exploited (and measured) as a

14 means of exploring temperature × CO_2 as well as temperature × CO_2 × O_3 interactions.

15   An indirect affirmation of the importance of temperature as a component of climate change

16 on wheat yield was provided by van Oijen and Ewart (1999), using two simulation models,

17 AFRCWHEAT2-O_3 and LINTULCC (Ewart et al., 1999). They analyzed data from the

18 ESPACE-wheat program, which involved 25 OTCs experiments in 1994, 1995, and 1996 at nine

19 European locations (Jäger et al., 1999). Both models were able to predict control-treatment grain

20 yields closely (5.5 ± 1.2 and 5.8 ± 1.2 t.ha^{-1}, respectively, versus the observed 5.9 ± 1.9 t.ha^{-1}),

21 and both indicated a predominantly negative effect of temperature on the yield response to

22 increased CO_2 (a 3 °C rise reduced the gain in yield from 30 to 14%). However, neither model

23 had an $R^2 > 0.3$, indicating that the models included other sources of variability among the sites

24 than the climatic factors. The multiple linear regression developed by Bender et al. (1999) based

25 on the same data sets also included temperature as a highly significant covariant. Both studies

26 are discussed more fully below.

27   Other studies, however, have found positive temperature-related growth effects, as

28 suggested by the early Idso and Idso (1994) analysis. In an OTC study using the perennial grass

29 *Festuca pratensis* in which a temperature increase of 3 °C above ambient was combined with

30 CO_2-enrichment to 700 ppm, both CO_2 and temperature caused increases in total above-ground

31 biomass (Hakala and Mela, 1996). Studies with potato (Cao et al., 1994) and soybean (Ziska and

1   Bunce, 1997) using potted plants in controlled environment chambers also showed temperature-

2   enhanced increases in growth in enriched CO_2 atmospheres. Read and Morgan (1996) compared

3   the effects of enriched CO_2 and temperature on two grasses: cool-season *Pascopyrum smithii*

4   and warm-season *Bouteloua gracilis*. In the latter (a C_4 species), 750 ppm CO_2 resulted in

5   increased dry matter production at daytime temperatures as high as 35 °C, but in *P. smithii*

6   (a C_3 species), CO_2-stimulated growth was greatest at 20 °C. However, the stimulation was

7   progressively attenuated by increased temperature, so that at 35 °C, growth in 750 ppm was only

8   one third of that in 350 ppm CO_2 at 20 °C.

9   Although the picture we have of temperature $\times$ CO_2 interactions is inconsistent, Rowland-

10   Bamford (2000) has provided persuasive evidence that the nature of response to temperature in

11   the grain yield of crops with as different temperature optima as rice and wheat will depend upon

12   whether the change is above or below the temperature optimum.

13   But what if we add O_3 as another variable? Unfortunately, there have been very few

14   studies of the three-way interaction. With the information available on CO_2 $\times$ O_3 interactions

15   (Table 9-12) and the limited information on temperature $\times$ O_3 interactions (discussed in Section

16   9.4.4.2) simulation modeling can attempt to provide estimates of O_3 $\times$ CO_2 $\times$ temperature effects,

17   but experimental observation is still required to validate the models. The questions that need to

18   be answered are: if increased temperature can offset the gains in productivity afforded by

19   increased CO_2 in important species such as wheat, and increased CO_2 can offset the reductions in

20   productivity caused by O_3, will increased temperature modify this protective effect. And if so, in

21   what manner.

22   To date, the only information available appears to consist of the reports by van Oijen and

23   Ewart (1999) and Bender et al. (1999) referred to above. In the former's simulation studies, the

24   overall yield depression of wheat caused by O_3 was found to be $7 \pm 4\%$ for both

25   AFRCWHEAT2-O_3 and LINTULCC models versus an observed $9 \pm 11\%$. The enhancements

26   due to CO_2 were predicted to be $24 \pm 9\%$ and $42 \pm 11\%$, respectively, which straddled the

27   observed $30 \pm 22\%$ gain. Based on the 13 experiments that included all four treatments ($\pm O_3$,

28   $\pm CO_2$), an actual 10% yield loss due to O_3 at ambient CO_2 levels was reduced to a 4% loss by the

29   elevated CO_2. The AFRCWHEAT2-O_3 model predicted 7 and 4% losses, and LINTULCC

30   model predicted 8 and 5% losses due to the O_3 and O_3 + CO_2 treatments. The actual and

31   simulated yield increases in response to CO_2 increased further with increasing temperature, but

1   although temperature had no discernible effect on the observed depression of yield caused by O_3

2   alone, both models suggested that the yield reduction was diminished both by higher

3   temperatures and higher CO_2 levels.

4   The analysis of the ESPACE-wheat experiments by Bender et al. (1999) led to the

5   following multiple linear regression:

6

7

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

9

10

11   where Y = grain yield, $g\text{-}m^{-2}$; $[CO_2]$ = ppm CO_2; $[O_3]$ = ppb O_3, 12-h mean; $[T]$ = °C; $[I]$ = light

12   intensity, $MJ\text{-}m^{-2}/day$; and $[H_2O]$ is a dummy variable: well watered = 1; limited water

13   supply = 2. (***, $p < 0.001$; **, $p < 0.01$; the coefficient for I was not significant.) With

14    $R^2 = 0.3983$, adjusted for 258 degrees of freedom, a large part of the variability was still

15   unaccounted for by the five variables. However, this analysis suggests that CO_2, O_3,

16   temperature, and water-status are important codeterminants of wheat yield but assumes no

17   interactions. Substitution in the model at summer light intensities and with well-watered plants

18   indicates that, at 20 °C, a doubling of CO_2 levels to 700 ppm alone, would lead to a 29.5%

19   increase in yield, while 50 ppb O_3 alone would decrease yield by 10.9%. With both gases at

20   those levels, the yield would only increase 20%, but with a concurrent temperature rise of 2 °C,

21   it would shrink to a 9.6% increase.

22   Both studies, therefore, indicate an amelioration of the effects of O_3 by CO_2, the magnitude

23   of which would be reduced at warmer temperatures. However, they relate to a single crop whose

24   response to CO_2 is temperature-sensitive. Information about other species in which the effects of

25   CO_2 and temperature are additive are limited. However, Wolf and Van Oijen (2003) recently

26   described a model (LPOTCO) simulating the effects of changes in climatic variables, CO_2 and

27   O_3 on tuber yield potential of irrigated potato (*Solanum tuberosum* cv. Bintje) over locations

28   within the European Union ranging from Finland to Italy. They noted that although increased

29   CO_2, O_3, and light intensity were predominant controlling factors, increased temperature also

30   influenced potential yields substantially, with increases in northern latitudes (attributed to a

1 longer growing season) but decreases in southern latitudes (attributed to decreased assimilate

2 production).

3   A clear understanding of the complex interactions of increased CO_2 and temperature with

4 O_3 must await further experimentation or simulations. However, it seems likely that any CO_2-

5 induced amelioration of the adverse effects of O_3 on aspects of growth other than seed or grain

6 yield may be lessened or increased by increased temperature, depending upon the temperature,

7 optima for the species, along the lines suggested by Rowland-Bamford (2000).

8   Other crop simulation models which incorporate O_3 and some of the various environmental

9 factors, including elements of climate change, have been reviewed by Kickert et al. (1999) and

10 Rötter and van de Geijn (1999). However, to date, the applications tend to have focused on

11 interactions of O_3 with factors such as soil moisture or nutrient availability.

12   With forest trees, the situation has the added complexity of a perennial growth form and

13 the inevitability, over time, of subjection to additional environmental stresses such as nutrient-

14 limitation. Here, too, although numerous models of tree growth have been described, there

15 appear to have been few applications to interactions of O_3 and factors of climate change.

16 Constable et al. (1996) used TREEGRO to model the growth of Ponderosa pine (*Pinus*

17 *ponderosa*) exposed to three O_3 levels (0.5×, 1.0×, and 2× ambient), two levels of CO_2 (ambient

18 and ambient + 200 ppm CO_2), and two temperature regimes (ambient and ambient + 4 °C). Plant

19 growth was predicted to be decreased 1, 19, and 39% by the three levels of O_3, respectively.

20 Increased CO_2 reduced the loss at the highest O_3 level to 7% but the combination of elevated

21 CO_2 with the higher temperature more than overcame the adverse effects of O_3, leading to a 4%

22 increase, largely attributed to increased fine root mass. The authors suggested that, in relation to

23 the baseline conditions used in the simulations (Corvallis, OR), higher concentrations of CO_2 and

24 O_3 and a warmer climate will have little impact on total-tree growth, but they noted the

25 importance of undertaking multiple stress studies in order to be able to make accurate forecasts

26 of the impact of such changes on forest trees.

27   More recently, Constable and Friend (2000) compared the capabilities of six published

28 process-based models (CARBON, ECOPHYS, PGSM, TRE-BGC, TREEGRO, and W91) for

29 simulating tree response to elevated CO_2, O_3, and temperature. They concluded that although

30 these models were capable integrators of the effects of various environmental factors on

1 individual processes such as photosynthesis, they were less reliable when extrapolating to

2 growth.

3 Although most of the research emphasis has been on simple $CO_2 \times O_3$ interactions, a few

4 isolated studies of interactions have involved O_3, CO_2, and biotic environmental factors. Heagle

5 et al. (1994) observed that both O_3 and CO_2 tended to be additive in encouraging the growth of

6 spider mite (*Tetranychus urticae*) populations on clover (*Trifolium repens*). Infection of wheat

7 (*Triticum aestivum*) with leaf rust (*Puccinia recondita*) sensitized the plants to O_3 injury, but its

8 severity was significantly reduced in elevated CO_2 (Tiedemann and Firsching, 2000). The

9 effects of O_3 and CO_2 on mycorrhizal symbioses was studied by Kytöviita et al. (1999) who

10 found that CO_2 did not ameliorate the adverse effects of O_3 on the root growth of Aleppo pine

11 (*Pinus halepensis*) and birch (*Betula pendula*). In another study with Aleppo pine, Kytöviita

12 et al. (2001) noted that both O_3 and elevated CO_2 reduced mycorrhiza-induced N-uptake by the

13 roots. In Scots pine (*Pinus sylvestris*), Kasurinen et al. (1999) observed transient effects of

14 elevated CO_2 and O_3 on root symbiosis, but none of the effects persisted over the 3 years of

15 the study.

16 The soil water $\times O_3 \times CO_2$ interaction was experimentally investigated by Broadmeadow

17 and Jackson (2000) in Durmast oak (*Quercus petraea*), European ash (*Fraxinus excelsior*), and

18 Scots pine (*Pinus sylvestris*). No interactions were noted with ash and pine; but with oak,

19 elevated CO_2 ameliorated and irrigation exacerbated the effects of O_3, although the resultant

20 effects were essentially additive.

21 Booker (2000) noted that soil nitrogen levels interacted only slightly with O_3 and CO_2 in

22 determining the composition of cotton (*Gossypium hirsutum*) leaves and roots. Carbon dioxide

23 reversed the inhibition of leaf growth caused by O_3, but increased N-fertility tended to reduce

24 this reversal.

25 Because of the small number of studies of possibly significant interactions of three or more

26 environmental factors, it impossible to draw any sweeping conclusions as to how O_3, in the

27 context of global climate change, may affect relationships among plants and insects, diseases,

28 and symbionts or among plants and nutrients or other air pollutants. The only interaction that

29 has some degree of general support is the amelioration of adverse O_3 effects by elevated CO_2.

30

1   **9.4.8.2   Ozone-UV-B Interactions**

2   As noted in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), depletion

3   of stratospheric O_3 by halofluorocarbons has resulted in increased intensities of ultraviolet-B

4   (UV-B) radiation (280 to 320 nm wavelengths) at the earth's surface. The situation is discussed

5   more fully in Chapter 10.

6   While stratospheric O_3 depletion may result in increased surface UV-B irradiation,

7   absorption of UV-B is a property of the O_3 molecule regardless of its location; and surface UV-B

8   flux is, therefore, also reduced by O_3 in the troposphere. Although only about 10% of the total

9   atmospheric O_3 column occurs in the troposphere (Fishman et al., 1990), it contributes a

10   disproportionately greater absorption effect than stratospheric O_3 because the UV radiation

11   penetrating the troposphere becomes increasingly diffuse as it reaches the surface, with a

12   consequent increase in mean path length (Brühl and Crutzen, 1989). Any benefits to vegetation

13   from reduced ambient O_3 stress must, therefore, also be viewed in the context of possible

14   adverse effects due to increased UV-B irradiation. There are, thus, two distinct types of possible

15   interactions between surface level O_3 and UV-B radiation:

16   • direct interactions involving simultaneous, sequential, or mixed exposures to O_3 and
       UV-B stresses; and

17   • effects on responses to UV-B itself resulting from changes in radiation intensity caused
       by changes in surface level O_3 concentrations.

18   Only the first type of interaction is discussed here. The second type of interaction has broad

19   implications for both health and welfare and focuses on the impacts of UV-V radiation per se.

20   It is dealt with separately in Chapter 10, which includes a critical review of the experimental

21   difficulties faced in undertaking meaningful plant research in order to reach a clear

22   understanding of the effects of increased UV-B radiation, the evidence for both its adverse and

23   beneficial effects on plants, and the potential for changes in ambient surface O_3 levels to modify

24   these effects.

25   The most recent reviews specifically addressing the *combined* effects of tropospheric O_3

26   and UV-B on plants are by Runeckles and Krupa (1994) and Krupa and Jäger (1996), although

27   the topic has also been included in several more general reviews of O_3 effects and factors of

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

29   (1998), Groth and Krupa (2000), and Krupa and Groth (2000).

1   However, little new information has become available since Runeckles and Krupa (1994)

2   noted that the scanty knowledge of the effects of UV-B and O_3 combinations available at that

3   time was derived solely from studies of soybean (*Glycine max*). Miller et al. (1994) observed no

4   interaction and no effect of UV-B on yield, in contrast to a previous report by Teramura et al.

5   (1990) using the same cultivar, Essex. More recently, in a study of the saltmarsh grass *Elymus*

6   *athericus* subjected to reciprocal exposures to O_3 and UV-B, van de Staaij et al. (1997) observed

7   no interactive effects and no adverse effects of UV-B following 14-day exposures, even though

8   an earlier report showed that longer exposures to UV-B (65 days) could cause a 35% loss of

9   biomass (Van De Staaij et al., 1993). However, in a study in which ambient, high altitude UV-B

10   levels were compared with near zero levels, at ambient or 2×-ambient levels of O_3, interactions

11   involving the levels of antioxidants in Norway spruce (*Picea abies*) and Scots pine (*Pinus*

12   *sylvestris*) were reported by Baumbusch et al. (1998). Schnitzler et al. (1999) subsequently

13   reported that O_3-induced injury and adverse effects on photosynthesis were more pronounced

14   with near zero UV-B levels, indicating an amelioration of the O_3-response. A later study with

15   Scots pine (Zinser et al., 2000) revealed $O_3 \times$ UV-B interactions at the gene expression and

16   biochemical levels. In contrast, Ormrod et al. (1995) reported that UV-B predisposed

17   *Arabidopsis thalliana* to injurious growth effects of O_3.

18   At various organizational levels, Runeckles and Krupa (1994) identified several similarities

19   between plant response to O_3 and UV-B, and at the level of gene expression there have recently

20   been several reports of both similarities and distinctions. Willekens et al. (1994) reported similar

21   effects of O_3, UV-B, and SO_2 on the expression of antioxidant genes in *Nicotiana*

22   *plumbaginifolia*. In parsley (*Petroselinum crispum*), Eckey-Kaltenbach et al. (1994a) found that

23   O_3 was a cross-inducer for both the UV-B-induced enhanced biosynthesis of flavonoids and the

24   pathogen-induced furanocoumarin phytoalexins, in keeping with the previously observed

25   O_3-induction of fungal and viral defense reactions. In this regard, Yalpani et al. (1994) provided

26   evidence that, in tobacco (*Nicotiana tabacum*), O_3 and UV-B acted similarly in increasing

27   disease-resistance via a salicylate-mediated enhancement of defense proteins. However,

28   subsequent work with tobacco led Thalmair et al. (1996) to conclude that exposure to UV-B did

29   not lead to the accumulation of pathogenesis-related proteins. In Scots pine (*Pinus sylvestris*),

30   although O_3 is known to induce stilbene synthase and cinnamyl alcohol dehydrogenase, UV-B

1 was found to enhance the former but suppress the latter, revealing an interaction at the level of

2 gene expression (Zinser et al., 2000).

3   In summary, the present base of information about possible interactions between increased

4 UV-B radiation and O_3 is insufficient to draw any firm conclusions in terms of gross effects, but

5 there is some evidence of similarities in the effects of O_3 and UV-B individually and of the

6 mechanisms involved at the molecular level.

7

8 **9.4.8.3   Interactions of Ozone with Multiple Climate Change Factors**

9   Despite of the need for experimental investigations of three-way or more complex

10 interactions among O_3, CO_2, UV-B, temperature, and other climate change factors, few studies

11 have been reported, even without O_3 as a factor. In an isolated report, using tomato

12 (*Lycopersicon esculentum*) seedlings, Hao et al. (2000) employed preexposure to UV-B ($\pm CO_2$

13 enrichment) followed by exposure to O_3 ($\pm CO_2$ enrichment). They observed that CO_2 more than

14 overcame the inhibition of photosynthesis caused by O_3, but pretreatment with UV-B reduced

15 the resultant increase.

16   In view of the unexpected observations made in their grassland study of the combined

17 effects of CO_2, temperature, precipitation, and N-supply, Shaw et al. (2002) affirmed that:

18 "Ecosystem responses to realistic combinations of global changes are not necessarily simple

19 combinations of the individual factors." The addition of O_3 to the list of variables results in

20 further complexity.

21   Although computer simulation modeling may ultimately lead to improved understanding of

22 these complex issues, to date, no such models appear to have been applied to these interactions,

23 possibly because of the scarcity of experimental data for parameterization.

24

25 **9.4.9   Summary - Environmental Factors**

26   Although O_3 and other photochemical oxidants are phytotoxic, their actions on vegetation

27 may be modified by a host of biotic and abiotic factors in the environment; conversely, they may

28 modify plant response to these other factors. The extensive review of these biological, physical,

29 and chemical factors conducted for the 1996 O_3 AQCD (U.S. Environmental Protection Agency,

30 1996) concluded with a statement that our understanding was too fragmented to permit drawing

31 many general conclusions. With today's increased awareness of the need for more complete

1   information on interactions, it is unfortunate that, in the interval since the 1996 criteria

2   document, rigorous, systematic investigations of interactions have been rare, and most of the

3   new information is as fragmented as before. This is inevitable partly in view of the vast scope of

4   the possible interactions between O_3 and other environmental variables. But it is also an

5   unavailable outcome of having the personal interests (and funding) of individual groups

6   determine the studies to be undertaken, instead of a coordinated program of research focused on

7   systematic investigations to improve our understanding our ability to assess the risks posed by

8   photochemical oxidants to cultivated and natural vegetation.

9   In the area of biotic interactions, new evidence with regard to insect pests and diseases has

10   done little to remove the uncertainties noted in the 1996 criteria document. Most of the large

11   number of such interactions that may affect crops, forest trees, and other natural vegetation have

12   yet to be studied. The trend suggested previously that O_3 increases the likelihood and success of

13   insect attack has received some support from recent studies, but only with respect to chewing

14   insects. With the economically important group of sucking insects such as the aphids, no clear

15   trends have been revealed by the latest studies. Hence, although it seems likely that some insect

16   problems could increase as a result of increased O_3 levels, we are still far from being able to

17   predict the nature of any particular O_3 plant insect interaction, its likelihood, or its severity.

18   The situation is a little clearer with respect to interactions involving facultative,

19   necrotrophic plant pathogens, with O_3 generally leading to increased disease. With obligate,

20   biotrophic fungal, bacterial, and nematode diseases, there are twice as many reports indicating

21   O_3-induced inhibitions than enhancements. The frequent reports that infection by obligate

22   biotrophs reduces the severity of O_3-induced foliar injury should not be interpreted as

23   "protection" because of the negative effects on the host plant of the disease per se. With obligate

24   biotrophs, the nature of any interaction with O_3 is probably dictated by the unique, highly

25   specific biochemical relationships between pathogen and host plant. At this time, therefore,

26   although some diseases may become more widespread or severe as a result of exposure to O_3, it

27   is still not possible to predict which diseases are likely to present the greatest risks to crops and

28   forests.

29   Several studies have indicated that the functioning of tree root symbioses with mycorrhizae

30   may be adversely affected by O_3, but there is also evidence that the presence of mycorrhizae may

31   overcome root diseases stimulated by O_3 and that O_3 may encourage the spread of mycorrhizae

1   to the roots of uninfected trees. The latest studies, therefore, present no clearer picture of the

2   likely nature of simple interactions of O_3 and root symbionts, but in view of the importance of

3   mycorrhizae as below-ground components of ecosystems, they are discussed more fully in

4   Section 9.5.

5   The few recent studies of the impact of O_3 on intraspecific plant competition have again

6   confirmed that grasses frequently show greater resilience than other types of plants. In grass-

7   legume pastures, the leguminous species suffer greater growth inhibition. And the suppression

8   of Ponderosa pine seedling growth by blue wild-rye grass was markedly increased by O_3.

9   However, we are far from being able to predict the outcome of the impact of O_3 on specific

10 competitive situations, such as successional plant communities or crop-weed interactions.

11   Light, a component of the plant's physical environment, is an essential "resource" whose

12 energy content drives photosynthesis and CO_2 assimilation. It has been suggested that increased

13 light intensity may increase the sensitivity to O_3 of light-tolerant species while decreasing that of

14 shade-tolerant species, but this appears to be an over-simplification with many exceptions.

15 Temperature affects the rates of all physiological processes based on enzyme-catalysis and

16 diffusion, and each process and overall growth (the integral of all processes) has a distinct

17 optimal temperature range. Although some recent field studies have indicated that O_3 impact

18 significantly increases with increased ambient temperature, other studies have revealed little

19 effect of temperature. But temperature is unquestionably an important variable affecting plant

20 response to O_3 in the presence of the elevated CO_2 levels contributing to global climate change

21 (see below). In contrast, evidence continues to accumulate to indicate that exposure to O_3

22 sensitizes plants to low temperature stress by reducing below-ground carbohydrate reserves,

23 possibly leading to responses in perennial species ranging from rapid demise to impaired growth

24 in subsequent seasons.

25   Although the RH of the ambient air has generally been found to increase the adverse

26 effects of O_3 by increasing stomatal conductance and thereby increasing O_3 flux, abundant

27 evidence indicates that the ready availability of soil moisture results in greater sensitivity to O_3.

28 The partial "protection" against the adverse effects of O_3 afforded by drought has been observed

29 in field experiments and modeled in computer simulations. There is also compelling evidence

30 that O_3 can predispose plants to drought stress. Hence, the response will depend to some extent

31 upon the sequence in which the stresses occur, but, even though the nature of the responses is

1 largely species-specific, successful applications of model simulations led to larger-scale

2 predictions of the consequences of O_3 × drought interactions. However, it must be recognized

3 that regardless of the interaction, the net result on growth in the short-term is negative, although

4 in the case of tree species, other responses such as increased water use efficiency could be a

5 benefit to long-term survival.

6   Mineral nutrients in the soil, other gaseous air pollutants, and agricultural chemicals

7 constitute chemical factors in the environment. The evidence regarding interactions with

8 specific nutrients is still contradictory. Some experimental evidence indicates that low general

9 fertility increases sensitivity to O_3, while simulation modeling of trees suggests that nutrient

10 deficiency and O_3 act less than additively, but there are too many example of contrary trends to

11 permit any sweeping conclusions. Somewhat analogously with temperature, it appears that any

12 shift away from the nutritional optimum may lead to greater sensitivity, but the shift would have

13 to be substantial before a significant effect on response to O_3 was observed.

14   Interactions of O_3 with other air pollutants have received relatively little recent attention.

15 The situation with SO_2 remains inconsistent, but seems unlikely to pose any additional risk to

16 those related to the individual pollutants. With the NO and NO_2, the situation is complicated by

17 their nutritional value as a N source. In leguminous species, it appears that NO_2 may reduce the

18 impact of O_3 on growth, with the reverse in other species, but the nature of the exposure pattern,

19 i.e., sequential or concurrent, also determines the outcome. Much more investigation is needed

20 before we will be able to predict the outcomes of different O_3-NO-NO_2 scenarios. The latest

21 research into O_3 × acid rain interactions has confirmed that, at realistic acidities, significant

22 interactions are unlikely. A continuing lack of information precludes offering any

23 generalizations about interactive effects of O_3 with NH_3, HF, or heavy metals. More evidence

24 has been reported that the application of fungicides affords some protective effects against O_3.

25   Over the last decade, considerable emphasis has been placed on research into O_3

26 interactions with the components of global climate change: increased atmospheric CO_2,

27 increased mean global temperatures, and increased surface level UV-B radiation. However,

28 most of these studies have tended to regard increased CO_2 levels and increased mean

29 temperatures as unrelated phenomena. Experiments into the effects of doubled CO_2 levels at

30 today's mean ambient temperatures are not pariculary helpful in trying to assess the impact of

31 *climate change* on responses to O_3. To date, the limited experimental evidence and evidence

1   obtained by computer simulation suggest that in a 600+ ppm world, although the enriched CO_2

2   would more than offset the impact of O_3 on responses as varied as wheat yield or the growth of

3   young Ponderosa pine trees, the concurrent increase in temperature would reduce but probably

4   not eliminate the net gain. A similar decrease in the net gain resulting from the complete

5   reversal by CO_2 of the inhibition of photosynthesis caused by O_3 has been reported for increased

6   UV-B irradiation. However, these are preliminary results based on minimal data.

7   In conclusion, although the increased use of computer simulations may be important in

8   suggesting outcomes of the many complex interactions of O_3 and various combinations of

9   environmental factors, the results obtained will only be as reliable as the input data used for their

10   parameterization. The data needed for good simulations can only come from organized,

11   systematic study. For predicting the future, ignorance is as good as dependence on poor

12   simulations.

13

14

15   **9.5   EFFECTS-BASED AIR QUALITY EXPOSURE- AND**
16   **   DOSE-RESPONSE INDICES**

17   **9.5.1   Introduction**

18   An index is needed that relates measured plant damage (i.e., growth) to ambient ozone

19   concentration over time. The index can provide both a consistency for reviewing exposure-

20   response effects in research, as well as a metric for developing a biologically-relevant air quality

21   standard that protects ecological resources. The quantifying function over some time frame has

22   frequently been referred to as "dose-response" and "exposure-response". The distinction being

23   where the pollutant concentration is measured: "Dose" is the measure of the pollutant

24   concentration absorbed by the leaf over some time period, whereas "exposure" is the ambient air

25   concentration measured nearby the plant over some time period.

26   Plant ozone uptake from the ambient air (either rate or uptake or cumulative seasonal

27   uptake) is the ideal measure, because without ozone or its reactive product(s) reaching the target

28   tissue there is no effect. Uptake is controlled in part by stomata (see Section 9.3 for detailed

29   discussion). An uptake measure should integrate all those environmental factors that influence

30   stomatal conductance, e.g., temperature, humidity, soil water status. However, a direct measure

31   of the internal leaf concentration of ozone is technically difficult and thus uptake values are

1 generally obtained with models that require species- and site-specific variables. Because of this,

2 a surrogate for uptake (i.e., exposure index) was sought early on using statistical summaries of

3 ambient pollutant concentration over some integral of time (O'Gara, 1922; Lefohn and Benedict,

4 1982; Lee et al., 1988; U.S. Environmental Protection Agency, 1986, 1992, 1996).

5   An index of exposure that is biologically relevant must then consider those factors known

6 to modify the plant response by altering ozone uptake (Hogsett et al., 1988; U.S. Environmental

7 Protection Agency, 1996), including the temporal dynamics of exposure (e.g., concentration,

8 frequency, duration), plant phenology (see Section 9.4), plant defense mechanisms (e.g.,

9 antioxidants) (see Section 9.3), and site climate and soil factors (e.g., temperature, vpd, soil

10 moisture) (see Section 9.4). In using these indices to develop air quality standards, the needs of

11 policy makers must also be considered, and those include simplicity and understandability

12 (Fairley and Blanchard, 1991). The development of such indices continues to be a challenge.

13

14   9.5.2   Summary of Conclusions from the Previous Criteria Document

15   The 1996 AQCD (U.S. Environmental Protection Agency, 1996a) focused primarily on

16 development of exposure indices, not flux, to quantify growth and yield effects in crops,

17 perennials and trees (primarily seedlings), and not foliar injury. The testing of the adequacy of

18 these indices to order the measured responses of growth and/or yield in crops and tree species, as

19 seedlings, was accomplished through regression analyses of earlier exposure studies. No direct

20 experimental testing of the range of indices has yet been accomplished. It was recognized that

21 these indices were only surrogates for O_3 uptake or dose. Their development focused on

22 consideration and inclusion of some, but not all, factors that affect O_3 uptake and expression of

23 effects (e.g., Lee et al., 1988). The 1996 document (U.S. Environmental Protection Agency,

24 1996a) drew a number of conclusions that built on even earlier conclusions (U.S. Environmental

25 Protection Agency, 1986, 1992). These conclusions are still valid today based on a review of

26 research published since 1996.

27   Studies prior to 1996, and after, indicate that the components of exposure, including

28 concentrations, temporal dynamics (e.g., time of day of peak events), frequency of occurrence,

29 duration, and respite time, are integral to developing indices of exposure that relate to growth

30 response. Evidence from the few direct experimental studies of varying exposure components

31 indicate the importance of peak concentrations, occurrence, respite time and the importance of

1   cumulative the concentrations (Musselman et al., 1983, 1986, 1994; Hogsett et al., 1885; U.S.

2   Environmental Protection Agency, 1996a).

3   Exposure duration influences the degree of plant response. Single season, year-long or

4   multi-year experimental results indicate that greater yield losses occurred when plants were

5   exposed for the longer duration, and that a cumulative-type index was able to better describe the

6   relationship between exposure and yield. Those indices not considering duration, e.g., 7-h

7   seasonal mean concentration index, single peak event index, or the index that cumulates all

8   concentrations (i.e., SUM00), were unable to adequately describe the relationship. These single

9   event or mean-type indices do not consider the role of duration of exposure and focus either only

10   on the peak event or put too much focus on the lower hourly average concentrations (U.S.

11   Environmental Protection Agency, 1996a).

12   Higher hourly averaged concentrations had a greater affect on plant response. It was

13   concluded that cumulative indices that gave greater weight to higher concentrations relate well

14   with plant response (crops and tree seedlings) and order the treatment means in monotonically

15   decreasing fashion with increasing exposure, based on studies that apply two or more types of

16   exposure regimes with replicate studies of the same species. These indices include, among

17   others: SUM06, W126, AOT40 (U.S. Environmental Protection Agency, 1996a).

18   No studies before or after 1996, have enabled a discrimination among the various

19   weighted, cumulative indices. Various functional weighting approaches were used, including

20   allometric, sigmoid or threshold weighting, and compared for best statistical fit of the plant

21   growth or yield data; but no one functional weighting was favored. For use as an air quality

22   standard, however, the need for simplicity, understandability, and ease of monitoring, favored

23   the cumulative threshold-weighted index (SUM06) (U.S. Environmental Protection Agency,

24   1996a).

25   Since higher concentrations occur primarily during the daylight hours, those indices that

26   differentially weight higher concentrations give greater weight to daylight hour concentrations.

27   This is important since stomatal conductance is usually greatest during the daylight hours,

28   compared to concentrations at night when conductance is usually minimal. Peak concentrations,

29   however, do not occur throughout the day; thus the timing of the peak concentration and

30   maximum plant uptake is critical in determining plant response. An exposure index that

31   incorporated either the daily or seasonal temporal patterns of higher concentration occurrence

1   with the temporal pattern of individual species' stomatal conductance was not reported in the

2   prior 1996 review (U.S. Environmental Protection Agency, 1996).

3   The relative importance of cumulative peak concentrations (> 0.10 ppm) versus cumulative

4   mid-range concentrations (0.05-0.099 ppm) was questioned based on ambient field exposures of

5   sensitive species. Although controlled experiments provided important evidence that the higher

6   hourly average concentrations should be given greater weight than the mid-level values in

7   developing indices, there was concern that under ambient conditions in the field the higher

8   concentrations did not occur at the time of maximum plant uptake. This coincidence was

9   considered to be the critical factor in determining peak concentration impacts on plants. Based

10   on the evidence at that time, it was not possible to conclude whether the cumulative effects of

11   mid-range concentrations were of greater importance than those of peak hourly average

12   concentrations in determining plant response (U.S. Environmental Protection Agency, 1996a).

13   No direct experimental studies had addressed this question prior to 1996, nor have any since.

14   The composite exposure-response functions for crops and tree seedlings were derived from

15   single and multi-year exposure studies that used modified or simulated ambient exposure

16   profiles. These profiles were typified by episodic occurrence of a large number of high O_3

17   concentrations; and this type of pattern is prevalent in many but not all rural agricultural and

18   some forested areas in the United States. Selecting a concentration value from these crop and

19   seedling response models may result in an over or underestimation of growth effects if applied to

20   regions of the country where a different type of exposure pattern is prevalent (U.S.

21   Environmental Protection Agency, 1996a). A multi-component index was suggested that

22   combined the peak-weighted, cumulative index with the number of occurrences of hourly

23   averaged concentrations $\geq$ 0.10 ppm that might reduce the uncertainty associated with selecting

24   the exposure value for protection based on NCLAN type studies (Lefohn and Foley, 1992;

25   Musselman et al., 1994; U.S. Environmental Protection Agency, 1996a). No direct experimental

26   studies addressed this question prior to 1996, nor have any since.

27   Since 1996, additional research has focused on the time of day when the higher hourly

28   average concentrations occur, the time of day of maximum plant uptake, and the diurnal

29   variability of plant defense mechanisms, and various suggestions as to inclusion of these factors

30   in any one of the peak weighted cumulative exposure indices. A much broader literature has

1   focused since 1996 on developing indices using flux to relate plant response. These new

2   developments are discussed in the sections that follow.

3

4   **9.5.3 Use of Exposure Indices to Establish Exposure-Response Relationships**

5   Mathematical approaches for summarizing ambient air quality information in biologically

6   meaningful forms that can serve as surrogates for dose for O_3 vegetation effects assessment

7   purposes have been explored for more than 80 years (O'Gara, 1922; U.S. Environmental

8   Protection Agency, 1996). Several of the indices introduced have attempted to incorporate some

9   of the biological, environmental, and exposure factors (directly or indirectly) that influence the

10   magnitude of the biological response and contribute to observed variability (Hogsett et al.,

11   1988). In the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996a), the exposure

12   indices were arranged into 5 categories, including (1) One Event, (2) Mean, (3) Cumulative,

13   (4) Concentration Weighted, and (5) Multi-component and were discussed in detail (Lee et al.,

14   1988). Figure 9-16 illustrates how several of the indices weight concentration and accumulate

15   exposure.

16   The indices were developed with knowledge of O_3 modes of action, as well as the role of

17   the exposure components and their adequacy tested using earlier exposure studies, e.g., NCLAN

18   (U.S. Environmental Protection Agency, 1996a; Hogsett et al., 1988). Various components of

19   the exposure-response, including concentration, time of day, respite time, frequency of peak

20   occurrence, plant phenology, predisposition, etc., were weighted with various functions and

21   evaluated on their ability in ordering the regression of exposure vs. growth or yield response.

22   The statistical evaluations for each of these indices were accomplished using growth/yield

23   response data from many earlier exposure studies (e.g., NCLAN). This retrospective approach

24   was necessary because there have been very few studies specifically designed to test the

25   goodness of fit of the indices. The regression approach selects those indices that most properly

26   order and space the treatment means to optimize the fit of a linear or curvi-linear model. This

27   approach provides evidence for the best indices, albeit not as defensible as that from studies with

28   experimental designs and analyses that focus on specific components of exposure.

29   Most of the early retrospective studies reporting regression approaches use data from the

30   NCLAN program or from Corvallis, OR (Lee et al., 1987; Lee et al., 1988; Lefohn and Irving,

31   1988; Tingey et al., 1989) or use data collected in California (Musselman et al., 1988; U.S.
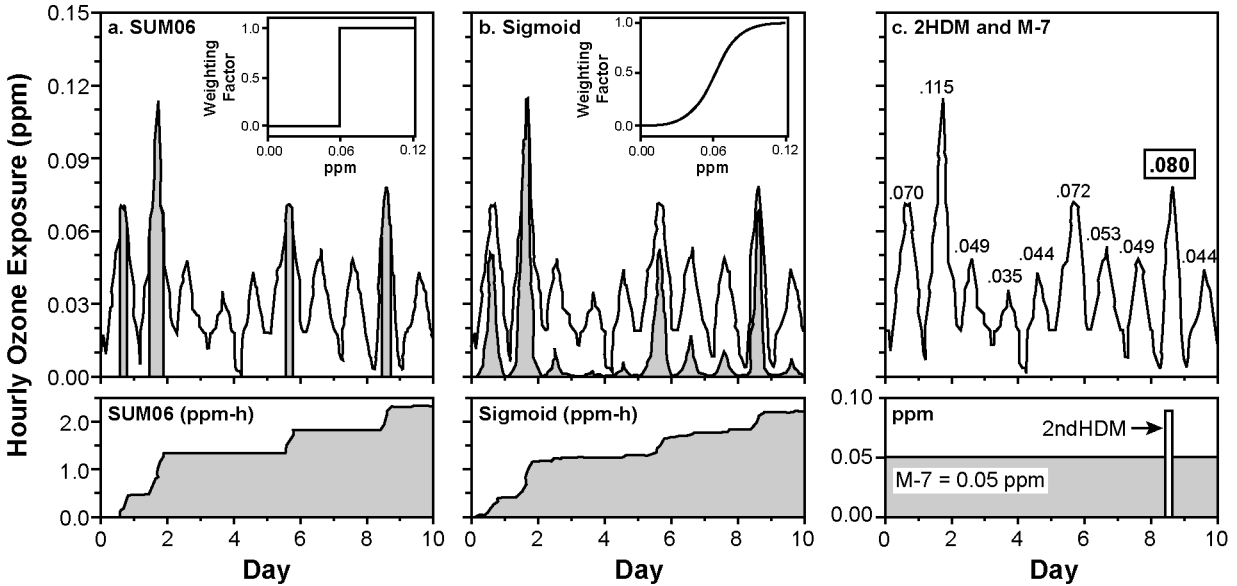
Figure 9-16. **Diagrammatic representation of several exposure indices, illustrating how they weight concentration and accumulate exposure. (a) SUM06 – The upper graphic illustrates an episodic exposure profile; the shaded area under some of the peaks illustrates the concentrations greater than or equal 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 mid-point 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: Tinge et al. (1991).

1   Environmental Protection Agency, 1986). These studies previously reviewed by EPA (U.S.

2   Environmental Protection Agency, 1992, 1996a) and were in general agreement and consistently

3   favored the use of cumulative peak-weighted exposure indices. Lee et al. (1987) suggested that

4   exposure indices that included all the data (24 h) performed better than those that used only 7 h

5   of data; this is consistent with the conclusions of Heagle et al. (1987) that plants receiving

1   exposures for an additional 5-h/day showed 10% greater yield loss than those exposed for

2   7-h/day. In a subsequent analysis using more of the NCLAN data, Lee et al. (1988) found the

3   "best" exposure index was a phenologically weighted, cumulative index, with sigmoid weighting

4   on concentration and a gamma weighting function as a surrogate for plant growth stage. This

5   index was the best statistical fit, but it depended upon more knowledge of species and site

6   conditions that made specification of weighting functions difficult for general use. This type of

7   multi-function index did not meet the other criteria for developing an air quality standard, that

8   being simplicity and understandability (Blanchard and Farley, 1999).

9   The next best fits were the several indices which only cumulated and weighted higher

10   concentrations (e.g., SUM06, SUM08, AOT40, W126). Amongst this group it was not possible

11   to distinguish a single best fit (Lee et al., 1988). Similarly Lefohn et al., (1992) reported that it

12   was not possible to differentiate among the SUM00, SUM06, SUM08, and W126 exposure

13   indices because the indices were highly correlated with one another in the experiment. Others

14   have reported similar results when attempting to identify optimum exposure indices (Musselman

15   et al., 1988).

16   Other factors, including predisposition time (Hogsett et al., 1988; McCool et al., 1988) and

17   crop development stage (Heagle et al., 1991; Tingey et al., 2002), contribute to variation in the

18   biological response and suggest the need for weighting O_3 concentrations to account for

19   predisposition time and phenology. However, the roles of predisposition and phenology in

20   influencing plant response vary considerably with species and environmental conditions, so that

21   specification of a weighting function for general use in characterizing plant exposure is not

22   possible at this time.

23   In similar retrospective analyses, using data from the European Open-Top Chamber

24   Program, Finnan et al. (1997) confirmed that cumulative exposure indices which emphasize

25   higher O_3 concentrations are best related to plant response and that cumulative exposure indices

26   which use weighting approaches provide a better fit (U.S. Environmental Protection Agency,

27   1996a).

28   The main conclusions from 1996 regarding a biologically-relevant index based on ambient

29   exposure still hold true today. No information has come forth in the interim that alters those

30   conclusions, and in fact, some recent studies have further substantiated them. These key

31   conclusions can be restated as follows:

1   • ozone effects are cumulative;

2   • peak concentrations appear to be more important than lower concentrations in eliciting
         a response;

3   • plant sensitivity to O_3 varies with time of day and crop development stage;

4   • exposure indices that accumulate the O_3 exposure and preferentially weight the peaks
         yield better statistical fits to response than do the mean and peak indices;

1   • to avoid possible overestimation of vegetation effects when using NCLAN-type yield
         data in exposure-response models, the cumulative exposure index might be combined
         with number of hours of high hourly average concentrations over the time period.

2   Following the 1996 review process (U.S. Environmental Protection Agency, 1996a,b), the

3   EPA proposed an alternative form of the secondary NAAQS using a cumulative, concentration-

4   weighted exposure index, the SUM06, to protect vegetation from damage (Federal Register,

5   1997). The EPA considered three specific concentration-weighted indices: the threshold

6   weighted SUM06, the AOT40, and the sigmoid weighted W126 exposure index (U.S.

7   Environmental Protection Agency, 1996b). Both indices performed equally well in predicting

8   the exposure-response relationships observed in the crop and tree seedlings studies conducted

9   during the prior 20 years (Heck and Cowling, 1997). In the absence of research results that

10   differentiate the predictive power of these two forms, the EPA selected the SUM06 exposure

11   index as the form for the proposed secondary standard, recognizing its simplicity,

12   understandability, and ease of use (U.S. Environmental Protection Agency, 1996b). A 3-month,

13   12-hour SUM06 exposure index of 26.4 ppm hr was proposed (U.S. Environmental Protection

14   Agency, 1996b). The value represented the concentration level that would protect 50% of the

15   crop species from a 10% yield loss. The composite response model included 49 studies

16   comprising 13 crop species and 5 locations across the U.S. where the crops are grown (U.S.

17   Environmental Protection Agency, 1996a).

18   European scientists took a similar approach as the United States in developing indices

19   describing growth and yield loss in crops and tree seedlings, using open-top chambers with

20   modified ambient exposures, but many fewer crop species were employed in the European

21   studies. The European countries were seeking a scientific basis for control strategies to reduce

22   air pollution. They adopted the critical levels and loads approach, as per the UN Economic

23   Commission for Europe (UNECE). 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" (United Nations Economic Commission for Europe, 1988). Critical levels are set to prevent long-term injury and damage to the most sensitive elements of any ecosystem. They are used to map and identify areas in Europe in which the levels are exceeded and that information is then used to plan optimized and effect-based abatement strategies. As used by the UNECE, they are not air quality standards in the US sense, but they have been used as targets for planning reductions in sulfur and nitrogen emissions to protect ecological resources. The nature of the significant harmful effects is not specified in the original definition, which provides 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 semi-natural 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 levels being stated as the cumulative exposure (concentration x hours) over a cut-off concentration of 40 ppb (AOT40) (Fuhrer et al., 1997). The "Level I" critical level was used in the 1990s to map areas of exceedance, but analyses of many exposure studies led to the conclusion that the simple, exposure-based level leads to over-estimation of the effects in some regions and under-estimation in others (Fuhrer et al., 1997; Kärenlampi and Skärby, 1996). The main problem was that other environmental factors (vapor pressure deficit, water stress, temperature, and light) and variation in canopy height altered O_3 uptake and its effects.

A decision was made to work towards a flux-based approach for the critical level, with the goal of modeling O_3 flux-effect relationships for three vegetation types: crops, forests, and semi-natural vegetation. Progress has been made in modeling flux (e.g., Grunhage and Jager, 2003; Ashmore et al., 2004a; Ashmore et al., 2004b) and the Mapping Manual is being revised (Karlsson et al., 2003; Ashmore et al., 2004a; Ashmore et al., 2004b; Grennfelt, 2004). The revisions may include a flux-based approach for 3 crops: wheat, potatoes, and cotton; but, because of lack of flux data, a cumulative, concentration-based (AOTx) exposure index will remain for most crops, and for forests and semi-natural herbaceous vegetation (Ashmore et al., 2004; Jaeger and Grunhage, 2004).

9.5.4 Identifying Exposure Components That Relate to Vegetation Effects

The efficacy of exposure indices to predict biological response requires that researchers identify a relationship between exposure components and effects, as well as those environmental and site factors that control pollutant uptake by the plant. These relationships were identified and discussed in the 1996 review (U.S. Environmental Protection Agency, 1996a). A significant, but in some instances, unquantifed 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 and 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.

9.5.4.1 Role of Concentration

A significant role of higher concentrations was established earlier, based on several experimental studies (U.S. Environmental Protections Agency, 1996a). Recently Nussbaum et al., (1995) and Yun and Laurence (1999b) have added support for the important role that peak concentrations, as well as the pattern of occurrence, plays in plant response to O_3. Based on air quality data from 10 U.S. cities, three treatments of 4-week exposure to the same SUM06 value were constructed by Yun and Laurence (1999b). They used the regimes to explore effects of treatments with variable peak occurrence versus uniform peak occurrence during the exposure period. The authors reported that the peak exposures were important and that the same SUM06 resulted in very different foliar injury. 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 in foliar injury of white birch saplings, but growth reductions were found to be more related to cumulative exposure. Nussbaum et al. (1995) also found peak concentrations and the pattern of occurrence to be critical in 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 may 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

1   National Forest. However, over the past 15 plus years, significant reductions in the O_3 exposure

2   have occurred in the San Bernardino National Forest (Lee et al., 2003; Lefohn and Shadwick,

3   2000; Davidson, 1993; Lloyd et al., 1989). An illustration of this improvement in air quality is

4   shown by the 37 year history of ozone air quality at a site in the San Bernardino Mountains

5   (Figure 9-17) (Lee et al., 2003). The O_3 exposure increased from 1963 to 1979 concurrent with

6   increased population and vehicular miles, followed by a decline to present mirroring decreases in

7   precursor emissions. The pattern in exposure was evident in various exposure indices including

8   the cumulative concentration weighted (SUM06), as well as maximum peak event (1-h peak),

9   and the number of days having hourly averaged O_3 concentrations $\geq$ 95 ppb (i.e., the California

10   ozone standard). The number of days having hourly averaged O_3 concentrations $\geq$ 95 ppb

11   declined significantly from 163 days in 1978 to 103 days in 1997. The changes in ambient

12   ozone air quality for the site were reflected in the changes in the frequency and magnitude of the

13   peak hourly concentration and the duration of the exposure (Figure 9-17). Considering the role

14   of exposure patterns in determining response, the seasonal and diurnal patterns in hourly O_3

15   concentration did not vary appreciably from year to year over the 37-year period (Lee et al.,

16   2003).

17   The inference for a role of higher concentrations comes from both results of ground

18   measures of tree conditions on established plots and from model simulations. Across a broad

19   area of the San Bernardino National Forest, the Forest Pest Management (FPM) method of injury

20   assessment indicated an improvement of crown condition from 1974 to 1988; and the area of

21   improvement in injury assessment is coincident with an improvement of O_3 air quality (Miller

22   and Rechel, 1999). A more recent analysis of forest changes in the San Bernardino National

23   Forest using an expanded network of monitoring sites has verified significant changes in growth,

24   mortality rates, basal area, and species composition throughout the area since 1974 (Arbaugh

25   et al., 2003). A model simulation of ponderosa pine growth over the 40 year period in the

26   San Bernardino showed a significant impact of ozone exposure on tree growth and indicates

27   improved growth with improving air quality. The improvement in growth was assigned to

28   improved air quality, but no distinction was made regarding the relative role of mid-range and

29   higher hourly concentrations, only that improved growth tracked both decreasing SUM06,

30   maximum peak concentration and number of days of hourly O_3 $\geq$ 95 ppb (Tingey et al., 2004).

31
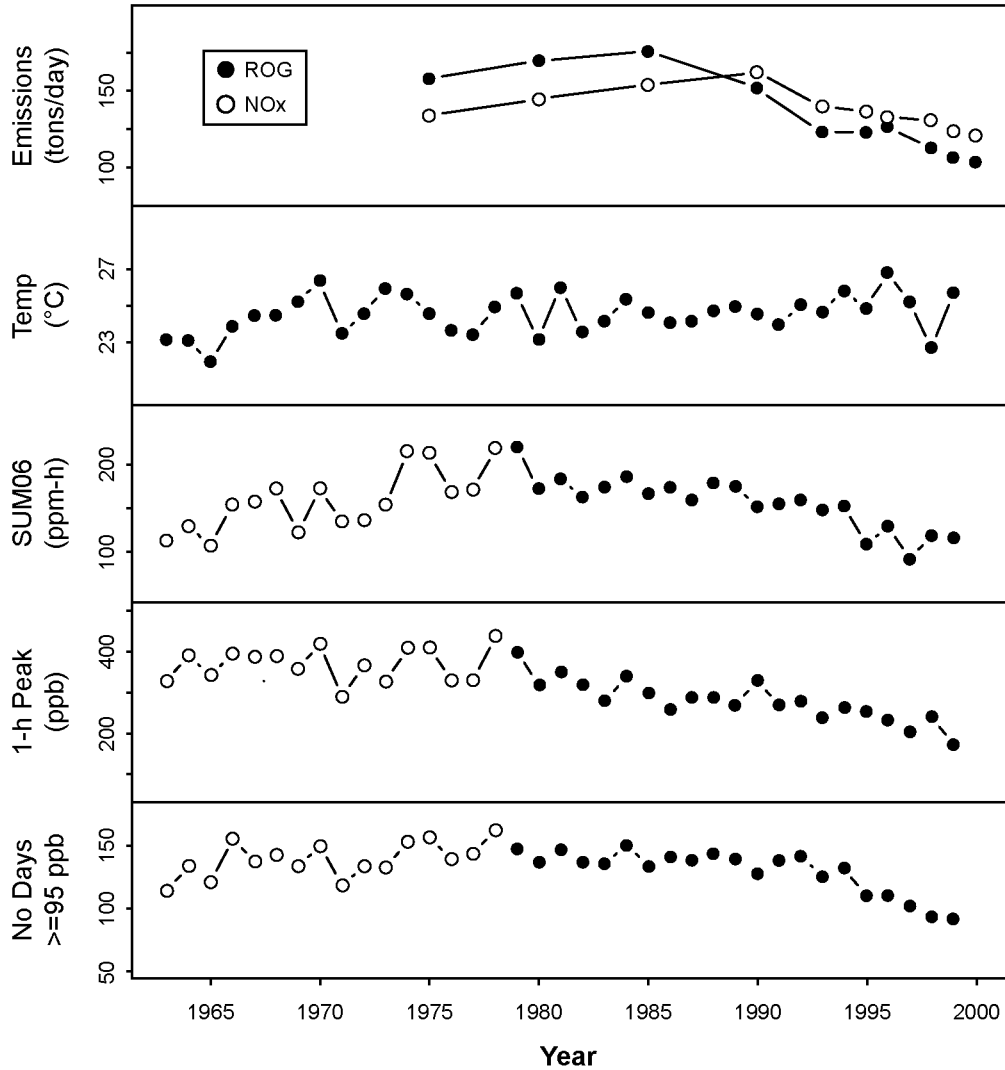
Figure 9-17. Trends in May-September 12-h SUM06, peak 1-h ozone concentration and number of daily exceedances of 95 ppb for Crestline in 1963-1999 in relation to trends in mean daily maximum temperature for Crestline and daily reactive organic gases (ROG) and oxides of nitrogen (NO$_x$) for San Bernardino county. Annual ROG and NO$_x$ emissions data for San Bernardino county were obtained from Alexis et al. (2001) and the California Air Resource Board's emission inventory available at http://www/arb/ca.gov/emisinv/emsmain/emsmain.htm.

Source: Lee et al. (2003).

1   **9.5.4.2 Role of Duration**

2   Recent studies have called into question the period of time over which concentrations are

3   accumulated and the form of the exposure index. Heagle and Stefanski (2000) reported that the

4   form of the exposure index was important only for 24-h indices for which SUM00 provided the

5   poorest fit. The authors reported that the SUM00, SUM06, W95 (Lefohn and Runeckles, 1987),

6   W126, and AOT40 produced similarly good fits of the foliage biomass data for 6-, 5-, and 4-h

7   midday accumulating periods. The study "pooled" data from San Bernardino (CA) and

8   Riverside (CA) with data from Amherst (MA), Corvallis (OR), Kennedy Space Center (FL),

9   Raleigh (NC), and Blacksburg (VA). Ozone exposures were much higher at the two California

10   sites (indicated by high W126, SUM06, W95, and AOT40 values) in comparison to the other

11   locations. Because of the pooling of the data, the large number of high hourly average O_3

12   concentrations that occurred at the California sites may have resulted in the exposure indices

13   being highly correlated with one another and made it difficult to identify one optimal index.

14   In another study in California, Arbaugh et al. (1998) reported that the SUM00 exposure

15   index performed better for describing visible injury than the SUM06, W126, number of hours

16   greater than or equal to 0.08 ppm, and the number of days between measurement periods. These

17   exposure indices were originally developed and tested using only growth/yield data, not foliar

18   injury (U.S. Environmental Protection Agency, 1996a). This distinction is critical in comparing

19   the efficacy of one index to another.

20   The SUM00 exposure index is a surrogate for a long-term average concentration and

21   earlier growth response studies indicated that a long-term average was not adequate in predicting

22   effects primarily because it gives equal weight to all concentrations (U.S. Environmental

23   Protection Agency, 1996). However, for many locations in California, a large number of higher

24   hourly average concentrations occur and the SUM00 could be highly correlated with the

25   frequency of elevated hourly average concentrations and thus could be a good predictor of

26   vegetation effects. In Section 9.3, it was noted that research results are mixed on what causes an

27   effect in plants: an event or the cumulation of events.

28

29   **9.5.4.3 Patterns of Exposure**

30   A significant factor in developing exposure indices is the temporal patterns of ozone

31   occurrence over a day, a month, a year, and seasonally overlaying the daily and seasonal

1   temporal patterns of those influential climatic and site factors. The coincidence of peak ozone

2   and maximal stomatal conductance and detoxification processes are key to effecting plant

3   growth response (Musselman and Minnick, 2000).

4

5   **Daily Patterns**

6   Recent experimental studies with tree species have demonstrated the de-coupling of

7   ambient O_3 exposure, peak occurrence, and gas exchange, particularly in areas of drought

8   (Panek, 2004). The coincidence of maximal conductance and occurrence of higher

9   concentrations are relevant to the time of day that exposure is cumulated in those indices that

10   cumulate and weight concentration.

11   The hours during the day over which ambient exposure is cumulated or effective dose is

12   estimated is a surrogate for the coincidence of these patterns of conductance and O_3 occurrence.

13   A 12-hr daylight period for cumulating exposure was proposed following the 1996 review (U.S.

14   Environmental Protection Agency, 1996). An extensive review of the literature revealed that a

15   large number of species had varying degrees of nocturnal stomatal conductance (Musselman

16   and Minnick, 2000). Grulke et al. (2003) showed that the stomatal conductance at night for

17   ponderosa pine in the San Bernardino (CA) National Forest ranged from one tenth to one fourth

18   that of maximum daytime gas exchange. In June, at the high-elevation site, 11% of the total

19   daily O_3 uptake of pole-sized trees occurred at night. In late summer, however, O_3 uptake at

20   night was negligible. Birch seedlings exposed to O_3 at night show greater reductions in growth

21   than those exposed to O_3 in daylight (Matyssek et al., 1995). *Brassica rapa* plants exposed to O_3

22   during the day or night, show little significant difference in the amounts of injury or reduced

23   growth response to treatment; the conductance was 70 to 80% lower at night (Winner et al.,

24   1989). Tissue biomass of ponderosa pine seedlings was significantly reduced when seedlings

25   were exposed to either daytime or nighttime episodic profiles (Lee and Hogsett 1999). However,

26   the biomass reductions were much greater with daytime peak concentrations than with nighttime

27   peak concentrations.

28   Although conductance is lower at night than during the day for most plants, nocturnal

29   conductance can result in some measurable O_3 flux into the plants and should be considered.

30   Nocturnal O_3 flux also depends on the level of turbulence that intermittently occurs at night.

31   In addition, plants can be more susceptible to O_3 exposure at night than during the daytime,

1　because of lower plant defenses at night (Musselman and Minnick, 2000). Massman (2004)

2　suggested that nocturnal stomatal O_3 uptake accounted for about 15% of the cumulative daily

3　effective O_3 dose that was related to predicted injury. Based on a review of the literature relating

4　to nocturnal stomatal conductance, Musselman and Minnick (2000) recommended that any O_3

5　exposure index used to relate air quality to plant response should use the 24-h cumulative

6　exposure period for both exposure-response and effective flux models.

7　　In general, stomatal conductance needs to be taken into account in developing indices

8　(Panek et al., 2002). Stomatal conductances are linked to both diurnal and seasonal

9　meteorological activity and soil/site conditions (e.g., soil moisture). Daily patterns of

10　conductance are often highest in mid-morning, whereas higher ambient O_3 concentrations

11　generally occur in mid to late afternoon, when stomata are often partially closed and

12　conductances are lower. Total flux depends on atmospheric resistance and boundary layer

13　resistances, both of which exhibit variability throughout the day. Several recent studies have

14　suggested that ponderosa pine trees in the southern and northern Sierra Nevada Mountains may

15　not be as sensitive to high O_3 concentrations as to lower concentrations, due to reduced O_3

16　uptake during the period when the highest concentrations occur (Panek et al., 2002; Panek and

17　Goldstein 2001; Bauer et al., 2000; Arbaugh et al.,1998). Panek et al. (2002) compared direct

18　measurements of ozone flux into a canopy of ponderosa pine and demonstrated a lack of

19　correlation of daily patterns of conductance and ozone occurrence, especially in the late season

20　drought period; and they concluded that a consideration of climate or season was essential,

21　especially considering the role of soil moisture and conductance/uptake. In contrast, Grulke

22　et al. (2002) reported high conductance when O_3 concentrations were high in the same species,

23　but different growing site conditions. The uncoupling of conductance and higher ambient O_3

24　concentration would hold true for more mesic environments as well as xeric landscapes. The

25　longer term biological responses reported by Miller and Rechel (1999) for ponderosa pine in the

26　same region and the general reduction in recent years in ambient O_3 concentrations, suggest that

27　conductance alone may not be a sufficient indicator of potential vegetation injury or damage.

28　　The generalized models of stomatal conductance may provide a means to link patterns of

29　O_3 occurrence with climatic and site factors that affect O_3 uptake to some degree, provided

30　conductance is modeled by regions of similar seasonal moisture and by similar canopy structure

31　(e.g., Emberson et al., 2000a,b; Grunhage 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 (Panek, 2004). These seasonal patterns can be early vs late season occurrence of higher O_3 concentrations, reflecting climate and precursor availability. The patterns also reflect seasonal drought and the role of soil moisture plays in stomatal conductance and O_3 uptake. Recently, studies have looked directly at this linkage. Panek et al. (2002) compared direct measurements of ozone flux into a canopy of ponderosa pine with a number of exposure indices, demonstrated a lack of correlation especially in the late season drought period, and concluded that a consideration of climate was essential, especially soil moisture. They suggested that a better metric for a seasonally drought-stressed forests would be one that incorporates forest physiological activity, either 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-September.

Using TREGRO, a process-based model, Tingey et al. (2004) simulated long-term growth of ponderosa pine over a 37-year period. The simulation showed high correlation between O_3 exposure and O_3-induced reductions in tree growth ($R^2 = 0.56$). The scatter about the line however indicates that other factors besides 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.

9.5.4.4 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, 1996a); and, since the last review, a few studies have corroborated the importance of this pattern in growth response. Köllner and Krause (2003) reported that, under equal exposure conditions, the most pronounced effects on the yield of sugar beet and soybeans occurred with those regimes that emphasized the

1   episodic occurrence of peak events. Nussbaum et al. (1995) compared the effects of different

2   patterns of peak occurrences with similar AOT40 values and reported a stronger effect on total

3   forage yield from the episodic treatment.

4

5   **9.5.4.5 Canopy Structure**

6   Another factor important in either O_3 exposure or uptake, is how canopy structure affects

7   O_3 concentration in and under forest canopies. There have been several comprehensive studies

8   of O_3 concentrations under tree canopies (Enders, 1992; Fontan et al., 1992; Fredericksen et al.,

9   1995; Joss and Graber, 1996; Kolb et al., 1997; Lorenzini and Nali, 1995; Neufeld et al., 1992;

10   Samuelson and Kelly, 1997). In general they indicate a reduction in O_3 of ~20 to 40% below the

11   canopy but above the shrub/herb layers. An essential component in the critical level AOT40 is

12   the height of where the O_3 concentration is measured. The critical levels are related to the O_3

13   concentration measured at the top of the canopy, i.e., upper surface boundary of the (quasi-)

14   laminar layer (Grunhage et al., 2003). This location is presumably more related to stomatal

15   uptake. Weighting the O_3 concentration at this location takes into account stomatal opening and,

16   if weighted with the Jarvis-Steward factors for radiation, temperature, and soil moisture, the

17   "toxicologically" effective AOT40 is obtained (Grunhage et al., 2003).

18   In a study that considers those factors important in O_3 uptake that are also spatially

19   distributed as a result of canopy structure, Davison et al. (2003) reported that the variation in

20   visible injury in coneflower populations is unlikely to be due to differences in O_3 flux and more

21   likely due to variation in PAR. At a height of 50 cm above ground, PAR was reduced by almost

22   90%, whereas the O_3 varied from about 15 to 90% of ambient. Ozone injury was not solely

23   related to O_3 flux. Although there have been studies of the effects of different light levels on O_3

24   response, there have been few at the very low levels that occur in canopies of tall herbaceous

25   stands or in the ground layer of forests. Davison et al. (2003) report that conductance was not

26   related to diurnal changes in light. The O_3 levels were still about 90% of the O_3 concentration

27   above the canopy when light was less than 5%. Light intensity dropped to 1.5% of open at 130

28   cm from the edge of the canopy, while O_3 dropped to only 42%. The study, although reporting

29   on the adequacy of visible foliar injury as an indicator of O_3 effects, does suggest that

30   consideration of other factors such as light are important in predicting response. How this may

31   be included in developing exposure-response indices was not considered.

1   **9.5.4.6 Site and Climate Factors**

2   Soil moisture is a critical factor in controlling O_3 uptake through it's effect on plant water

3   status and stomatal conductance.. In an attempt to relate uptake, soil moisture, and ambient air

4   quality to identify areas of potential risk, available O_3 monitoring data for 1983 to 1990 were

5   used, along with literature-based seedling exposure-response data from regions within the

6   southern Appalachian Mountains that might have experienced O_3 exposures sufficient to inhibit

7   growth (Lefohn et al., 1997). In a small number of areas within the region, O_3 exposures and

8   soil moisture availability were sufficient to possibly result in growth losses in some sensitive

9   species (e.g., black cherry). The conclusions were limited because of the interpolation of the O_3

10   exposures in many of the areas and the hydrologic index used might not reflect actual water

11   stress.

12

13   **9.5.4.7 Plant Defense Mechanism - Detoxification**

14   The non-stomatal component of plant defenses are the most difficult to quantify, but some

15   studies are available (Barnes et al., 2002; Plöchl et al., 2000; Chen et al., 1998; Massman and

16   Grantz, 1995). Massman et al. (2000) developed a conceptual model of a dose-based index to

17   determine plant injury response to O_3 that relates to the traditional exposure-based parameters.

18   The index uses time-varying-weighted fluxes to account for the fact that flux is not necessarily

19   correlated with plant injury or damage. Their model applies to plant foliar injury, and they

20   suggest that application of flux-based models for determining plant damage (yield or biomass)

21   will require a better understanding and quantification of the injury and damage relationship.

22

23   **9.5.5 Ozone Uptake or Effective Dose as an Index**

24   Developing an index that relates growth response to ambient exposure has been

25   approached in the past through various weighting functions on those ambient exposure factors,

26   including concentration, duration, and time of day for cumulating exposure. These indices have

27   not incorporated factors of climate patterns and species- and site factors that control O_3 uptake

28   via canopy and stomatal conductance. The other approach is basing the index on the O_3

29   concentration going into the leaf, or flux. This approach includes those factors controlling

30   uptake through canopy and stomatal conductance and, by necessity, relies on models to predict

31   flux and ultimately the "effective" flux: that concentration that reaches the target tissue to cause

1   an effect (Grunhage et al., 2004; Massman 2004; Massman et al., 2000) .Effective flux has been

2   defined as the balance between the O_3 flux and the detoxification process (Dämmgen et al.,

3   1993; Grunhage and Haenel, 1997; Musselman and Massman, 1999). The time-integrated

4   effective flux is termed "effective dose". The uptake mechanisms and the resistances in this

5   process, including stomatal conductance and biochemical defense mechanisms, are discussed in

6   the previous Section 9.3.

7

8   **9.5.5.1 Models of Stomatal Conductance**

9   Only a limited number of studies have measured O_3 concentration or its reaction products

10   within the leaf. Otherwise the index of uptake is a modeled result that considers site, climatic,

11   meteorological, and species-specific (e.g., detoxification reactions) factors. Models of O_3

12   conductance into plant tissue are available (Grunhage and Jager, 2003; Emberson et al., 2000a,b;

13   Grunhage and Haenel, 1997; Grünhage et al., 1997; Massman, 1993; Wesely, 1989). The

14   European Monitoring and Evaluation Program (EMEP) has an O_3 deposition model developed

15   for application across Europe in conjunction with the EMEP photochemical model as a tool for

16   the critical levels program. The model has been developed to estimate vegetation type-specific

17   O_3 deposition and stomatal flux, calculated according to a standard 3-resistance formulation

18   incorporating atmospheric, boundary layer, and stomatal resistances (Emberson 2000b). The

19   model uses a multiplicative algorithm of the stomatal conductance of O_3 (Jarvis, 1976) and has

20   been parameterized for 10 European tree species, 7 agricultural species, and 1 type of semi-

21   natural vegetation. The model calculates conductance as a function of leaf phenology,

22   temperature, photosynthetic flux density (PFD), vapor pressure deficit (VPD), and soil moisture

23   (SMD). The environmental variables are site-specific (or regionally-specific). The most

24   important factors limiting O_3 with this model were vapor pressure deficit (VPD), soil moisture

25   deficit, and phenology (Emberson et al., 2000b). These factors demonstrate the critical linkage

26   of high VPD and stomatal closure, which typically co-occurs with high O_3 concentrations.

27   A number of recent model-based studies relating flux and plant growth response have

28   investigated several crop and forest tree species (Karlsson et al., 2004; Karlsson et al., 2004;

29   Pleijel et al., 2004; Matyssek et al., 2004; Elvira et al., 2004; Soja et al., 2004; Weiser and

30   Emberson, 2004; Touvinen et al., 2004; Altimir, et al., 2004; Gerosa et al., 2004; Mikkelsen,

31   et al., 2004, Bassin, et al., 2004; Emberson et al., 2000). The studies have used earlier exposure

1   experiments as well as explicitly designed field studies but, to date, no abundance of data that

2   would provide the basis for a flux-based index.

3   The complexity of using flux as an index of O_3 exposure for response is shown in field

4   studies that measured O_3 flux into Norway spruce and cembran pine (Wieser et al., 2000). They

5   demonstrated that stomatal conductance was the main limiting factor for O_3 uptake and showed

6   the dependence of that measure on crown position, needle age, and altitude. Consideration of the

7   role of climate illustrates the importance of a flux measure. Pleijel et al., (2000) reported the

8   improved relationship of yield in spring and winter wheat grown in OTCs in many areas across

9   Europe when it was related to the cumulative stomatal O_3 uptake during the grain-filling period.

10 Compared to the AOT40, the cumulative uptake index estimated larger yield losses in the

11 relatively humid parts of western and northern Europe, while smaller yield loss was estimated

12 for the dry summer climates in south and central Europe.

13   Danielsson et al. (2003) compared the ability of two different stomatal models to relate

14 grain yield in field grown spring wheat to cumulated O_3 uptake and an exposure index of

15 AOT40, and found that the cumulated O_3 uptake determined with either model performed better

16 in relating exposure to yield than did the cumulative exposure index of AOT40.

17   Cumulative O_3 uptake was modeled for three deciduous and 2 confierous species growing a

18 different sites and elevations and compared with exposure measure of AOT40 at these sites

19 (Matyssek et al., 2004). A general linearity was demonstrated between the two measures of O_3

20 exposure; and, at any given AOT40, there was a $25 \pm 11\%$ variation in CU. Although no

21 correlation of growth alterations was observed with either the exposure or the uptake measure,

22 the modeled cumulative uptake was able to describe the variation in tree size and site location

23 which makes for a better measure in risk assessment of O_3 (Matyssek et al., 2004). Karlsson

24 et al. (2004) compared the biomass-response relationship in young trees at seven experimental

25 sites across Europe using modeled cumulative O_3 uptake and AOT40. A weaker dose-response

26 relationships were reported for the cumulative uptake metric than the AOT40 (Karlsson et al.,

27 2004),

28   Concern about the complexity of the stomatal models and the data needed to model O_3

29 uptake, has led some researchers to offer modified accumulated exposure indices that consider

30 those meteorological factors controlling uptake (Karlsson et al., 2004; Gerosa et al., 2004). In a

31 study of subterranean clover in Austria, Belgium, and southern Sweden, Karlsson et al., (2004)

1   reported on the performance of a modified accumulated exposure over the threshold (mAOT)

2   which was based on solar radiation and vapor pressure deficit. This index improved the

3   relationship for observed visible injury. But if modeled uptake of O_3 was derived from a simple

4   stomatal conductance model considering solar radiation, VPD, and air temperature, then this

5   index gave an even greater improvement in the relationship to visible injury than did the ambient

6   exposure index of AOT40 (Karlsson et al., 2004B). The added value of the mAOT was

7   worthwhile and it had a lower degree of complexity and data requirements modeling O_3 uptake

8   with stomatal models. Based on a study of O_3 fluxes over a barley field in Italy, a similar

9   modified exposure index was reported and referred to as "effective exposure" (Gerosa et al.,

10 2004). Their approach was similar in its consideration of physiological aspects in conjunction

11 with monitored O_3 concentrations. It addressed the shortcoming of the data needs for modeled

12 O_3 uptake.

13   Models that partition O_3 uptake into stomatal and non-stomatal components are also now

14 available and predict a significant non-stomatal component in calculating O_3 flux (Altimir et al.,

15 2004; Mikkelsen et al., 2004; Nikolov and Zeller, 2003; Zeller and Nikolov, 2000; Bassin et al.,

16 2004; Nussbaum et al., 2003). Altimir et al. (2004) compared the relative contribution of

17 stomatal and non-stomatal sinks at the shoot level for Scots pine. Using the EMEP model with a

18 revised parameterization for Scots pine, they demonstrated that a major removal of O_3 was due

19 to the non-stomatal component; and when a non-stomatal term was introduced dependent on

20 ambient relative humidity, the non-stomatal contribution to the total conductance was about

21 50%. Zeller and Nikolov (2000) demonstrated a large non-stomatal O_3 uptake (41% of the total

22 annual flux) in subalpine fir at a site in southern Wyoming using the biophysical model

23 FORFLUX. In a 5 year study of measured O_3 flux to a Norway spruce canopy, Mikkelsen et al.

24 showed monthly patterns of non-stomatal and stomatal deposition as part of total deposition to

25 the canopy. Their study demonstrated that daily means of O_3 concentration and fluxes averaged

26 over 5 years correlate well, but the correlation is based on two different uncoupled processes

27 outside and inside the stomates. The destruction of O_3 in the canopy outside the stomates is

28 influenced by temperature, light and humidity, e.g., surface reactions, NO- and VOC-emissions;

29 and these same factors influence stomatal opening, e.g., mid-day and night closure.

30 Consequently, the diurnal O_3 concentration and O_3 flux do not correlate at all during the growing

31 season. The study estimated yearly stomatal uptake to be a minimum of 21% of total deposition

1   (i.e., non-stomatal as high as 80% of total). The stomatal uptake is highest in May-August (30-

2   33%) and lowest in November-February (4-9%).

3

9.5.5.2 Nonlinear Response and Developing Flux Indices

5   If only O_3 flux were used as a metric to predict vegetation injury or damage, the prediction

6   might be overestimated because of reported non-linear relationships between O_3 and plant

7   response (Amiro et al., 1984; Amiro and Gillespie, 1985; Bennett, 1979; U.S. Environmental

8   Protection Agency, 1978; 1986;1996). The non-linearity in the response surface suggested the

9   existence of a biochemical threshold. More recently, nonlinear relationships between O_3 flux

10   and yield were shown for potato (Pleijel et al.,2002) and spring wheat (Danielsson et al.,2003).

11   The relationship between O_3 flux and potato yield suggested using an instantaneous flux

12   threshold to overcome the nonlinear relationship (Pleijel et al., 2002). However, the authors did

13   not report a substantial improvement in the mathematical fitting of the model when applying the

14   threshold Most of the flux was accumulated below 0.06 ppm. Danielsson et al. (2003) was able

15   to show an improved relationship between O_3 uptake and yield of spring wheat using a threshold

16   of 5 nmoles m^{-2} sec^{-1} (0.24 mg m^{-2} sec^{-1}). These results suggest not all O_3 entering the stomata

17   contribute to a reduction in yield, which depends to some degree on the amount of internal

18   detoxification occurring with each particular species (see Section 9.3). The fact that the defense

19   and repair mechanisms vary diurnally as well as seasonally may make it extremely difficult to

20   apply a mathematically determined threshold to instantaneous flux measurements to calculate

21   cumulative flux. The threshold models do not allow for the temporal (i.e., daily and seasonal)

22   variability of defense mechanisms.

23   Musselman and Massman (1999) suggest that those species having high amounts of

24   detoxification potential may show less of a relationship of O_3 stomatal uptake to plant response.

25   There has been no direct experimental demonstration of this relationship, however. The cellular

26   detoxification reactions and repair processes which both detoxify oxidants as well as play central

27   role in the carbon economy of the plant are another level of resistance to O_3 reaching the target

28   tissue (see Section 9.3). As indicated earlier, effects occur on vegetation when the amount of

29   pollutant absorbed exceeds the ability of the plant to detoxify O_3 or to repair the initial impact.

30   The magnitude of the response is determined by the amount of the pollutant reaching the target

31   site and the ability of the plant to reestablish homeostatic equilibrium. Thus, one would expect

1   to observe a decoupling of O_3 uptake with vegetation effects, which would manifest itself as a

2   nonlinear relationship between O_3 flux and injury or damage.

3   Additional factors for inclusion in flux-based models to predict vegetation effects are the

4   defense and repair mechanisms. Specifically, the relationship between conductance, O_3

5   concentration, and defense/repair mechanisms needs to be included. Recently, Massman (2004)

6   described results that illustrate that the combination of conductance, O_3 concentration, and

7   diurnal variation of defense mechanisms show the daily maximum potential for plant injury

8   (based on effective dose) coincides with the daily peak in O_3 mixing ratio. Massman et al.

9   (2000) stress that the product of the overlapping mathematical relationships of conductance,

10 concentration, and defense mechanisms results in a much different picture of potential impact to

11 vegetation than just the use of conductance and concentration in predicting vegetation effects.

12

13 **9.5.5.3 Simulation Models**

14   Another approach for determining O_3 uptake and relating growth response to ambient O_3

15 exposure may be the use of physiologically-based simulation models. Several of these have

16 been used in various contexts, comparing O_3 response in a number of tree species with varying

17 climate and site factors (e.g., soil moisture) (e.g., Laurence et al., 2001; Ollinger et al., 1997;

18 Ollinger et al., 1998; Weinstein et al., 2001; Weinstein et al., 2004; Tingey et al., 2000; Tingey

19 et al., 2004). One of the important considerations in applying simulation modeling is to

20 carefully assess the uncertainties associated with the modeling predictions. Further efforts need

21 to be made to exercise the models so that they predict past growth losses associated with changes

22 in O_3 exposures that can be verified with on-the-ground surveys.

23

24 ## 9.5.6 Summary

25   A few studies published since 1996 have substantiated earlier conclusions on the role of O_3

26 exposure components (including concentration, duration and exposure patterns) in describing

27 growth response to O_3 exposures. Recent studies of different exposure patterns have confirmed

28 earlier studies on the importance of higher concentrations and duration of exposure when

29 describing response. An inferred role of peak concentrations is possible from consideration of

30 improved air quality in regions like the San Bernardino Mountains in southern California.

1   Studies provide the basis for focusing on the higher O_3 concentrations, while including the lower

2   levels, when estimating the effects of emission reductions on vegetation.

3   A few studies have demonstrated the potential disconnection of peak events and maximal

4   stomatal conductance. In addition, a few studies have demonstrated the uptake of O_3 during

5   nighttime hours and suggested the need to cumulate O_3 exposure 24 h per day and not just during

6   daylight hours.

7   Several studies since 1996 have demonstrated another critical concern in developing an

8   index for exposure. The concern is that peak O_3 events and maximum stomatal conductance

9   may be temporally separate. This disconnection introduces uncertainty in assessing O_3 impact

10   when using the current ambient exposure based cumulative, concentration weighted indices.

11   If stomatal conductance is relatively low, as in the late afternoon in arid climates, and that is the

12   same time as the peak O_3 concentrations, then use of an exposure index that does not consider

13   this disconnect, will overestimate the effect of the exposure. This concern is especially apparent

14   when assessing the impact of O_3 across all the varied climatic regions of the United States or

15   Europe. Some studies use stomatal models to predict uptake or using process level models (e.g.,

16   TREGRO) to integrate those climate and site factors that drive this temporal pattern of stomatal

17   conductance and exposure, and thus reduce some of the uncertainty in regional to national

18   assessments of effects. These approaches however are still species dependent.

19   The results of these studies and reviews have indicated the need to continue to develop

20   indices that are more physiologically and meteorologically connected to the actual dose of O_3 the

21   plant receives. The cumulative concentration-weighted exposure indices are acknowledged

22   surrogates for effective dose that are simple conceptually and easy to measure. They do not fully

23   characterize the potential for plant uptake and resulting effects associated with O_3 because the

24   indices, being measures of ambient concentration, do not include the physical, biological, and

25   meteorological processes controlling the transfer of O_3 from the atmosphere through the leaf and

26   into the leaf interior. Use of such indices is especially limited in spatial risk characterizations

27   because of the lack of linkage of meteorology, species and site-specific factors influencing O_3

28   uptake. The flux-based approach should provide an opportunity to improve upon the

29   concentration-based (i.e., exposure indices) approach. A cautionary argument was advanced in a

30   few publications centered around the non-linear relationship between ozone uptake and plant

31   injury (not growth alterations) response. The concern was that not all O_3 stomatal uptake results

1 in a reduction in yield, which depends to some degree on the amount of internal detoxification

2 occurring with each particular species. Those species having high amounts of detoxification

3 potential may show less of a relationship between O_3 stomatal uptake and plant response.

4   The European approach and acceptance of flux-based critical values is a recognition of this

5 problem; and a concerted research effort is needed to develop the necessary experimental data

6 and modeling tools that will provide the scientific basis for such critical levels for O_3 (Grunhage

7 et al., 2004; Fuhrer et al., 1997; Grunhage and Jager, 1994).

8   At this time, based on the current state of knowledge, exposure indices that differentially

9 weight the higher hourly average O_3 concentrations, but include the mid-level values, represent

10 the best approach in the United States for relating vegetation effects to O_3 exposure.. A large

11 database exists that has been used for establishing exposure-response relationships. Such a

12 database does not yet exist for relating O_3 flux to growth response. Those pattern disconnects

13 between period of uptake and peak occurrence, as well as the potential for nocturnal uptake,

14 should be considered with some weighting functions in the currently used exposure indices.

15 Of particular consideration would be their inclusion in regional to national estimations of O_3

16 impacts on vegetation. Another useful approach to regional assessment for given specie(s) is

17 simulating growth effects with process-based models that account for seasonal climate and site

18 factors that control conductance

19   It is anticipated that, as the overlapping mathematical relationships of conductance,

20 concentration, and defense mechanisms are better defined, O_3 flux-based models may be able to

21 predict vegetation injury and/or damage at least for some categories of canopy-types with more

22 accuracy than the exposure-response models.

23

24

9.6 OZONE EXPOSURE-PLANT RESPONSE RELATIONSHIPS

9.6.1 Introduction

27   Ambient O_3 concentrations have long been known to cause visible symptoms, decreases in

28 photosynthetic rates, decreases in plant growth, and decreases in the yield of marketable organs

29 (U.S. Environmental Protection Agency, 1978, 1986, 1996). Yet, despite considerable research

30 in the U.S. and other countries during the past three decades, quantifying the effects of ambient

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

1   plant responses, with most effort focused on the yield of crops and the growth of tree seedlings.

2   Most experiments exposed individual plants grown in pots or soil under controlled conditions to

3   known concentrations of O_3 for a segment of daylight hours for some portion of the plant's life

4   span (Section 9.2). The response of a plant species or variety to O_3 exposure depends upon

5   many factors discussed in previous sections, including genetic characteristics (Section 9.4.2),

6   biochemical and physiological status (Section 9.4), and previous and current exposure to other

7   stressors (Sections 9.4, 9.5). Section 9.4 describes how O_3 moves from the atmosphere into the

8   leaf and the subsequent biochemical and physiological responses of plants. The current section

9   focuses on the quantitative responses of plants to seasonal or multiyear exposures to known

10   amounts of O_3. Quantitative responses include foliar symptoms and decreased growth of whole

11   plants or decreased harvestable portions of them. Because of the available information, most of

12   this section focuses on the response of individual plants, especially crop plants and tree

13   seedlings, with limited discussion of mixtures of herbaceous species. The responses of natural

14   ecosystems are discussed in Section 9.7.

15   This section will pay particular attention to studies conducted since the publication of the

16   1996 AQCD (U.S. Environmental Protection Agency, 1996). However, because much O_3

17   research was conducted prior to the 1996 AQCD, the present discussion of vegetation response

18   to O_3 exposure is largely based on the conclusions of the 1978, 1986, and 1996 criteria

19   documents (U.S. Environmental Protection Agency, 1978, 1986, 1996). To provide a context for

20   the discussion of recent research, the the key findings and conclusions of those three documents

21   are first summarized below.

22

23   **9.6.2   Summary of Key Findings/Conclusions from Previous Criteria**
24   **Documents**

25   Experimental data reviewed in the 1978 and 1986 criteria documents dealt primarily with

26   the effects of O_3 on agricultural crop species (U.S. Environmental Protection Agency, 1978,

27   1986). The chapter on vegetation effects in the 1978 document (U.S. Environmental Protection

28   Agency, 1978) emphasized foliar symptoms and growth effects, but not those effects that

29   affected yield, an emphasis dictated by the kind of data available at the time. The 1986

30   document reviewed a substantial new body of evidence based on open-top chamber (OTC)

31   experiments (see Section 9.2) showing that ambient O_3 exposures reduced the growth and yield

1   of herbaceous plants, again with a focus on major crop species. In the 1986 and 1996

2   documents, data were presented from regression studies conducted to develop exposure-response

3   functions for estimating yield loss of major crop species in different regions of the United States.

4   The 1996 document included results from additional herbaceous crop species as well as shrub

5   and tree species. For a number of tree species, biomass growth of seedlings was related to

6   growing season O_3 exposures to produce response functions for estimating O_3 exposures that

7   reduce growth by 10 or 30%. Also, in the 1986 and 1996 documents, data from studies using

8   ethylene diurea (EDU) as a protectant were reviewed. The 1978, 1986, and 1996 criteria

9   documents also reviewed data on the response to O_3 exposures of forest ecosystems in the

10 San Bernardino Mountains of southern California (U.S. Environmental Protection Agency, 1978,

11 1986, 1996). Because this region is exposed to high concentrations of O_3 and has shown

12 evidence of ecosystem-level changes, it remains an important study area (see Section 9.7).

13   Ozone can cause a range of effects, beginning with individual cells, leaves, and plants, and

14 proceeding to plant populations and communities. These effects may be classified as either

15 injury or damage. Injury encompasses all plant reactions, such as reversible changes in plant

16 metabolism (e.g., altered photosynthetic rate), altered plant quality, or reduced growth that does

17 not impair yield or the intended use or value of the plant (Guderian, 1977). In contrast, damage

18 includes all effects that reduce or impair the intended use or value of the plant. Damage includes

19 reductions in aesthetic values as well as losses in terms of weight, number, or size of the plant

20 part that is harvested (yield loss). Yield loss also may include changes in crop quality, i.e.,

21 physical appearance, chemical composition, or the ability to withstand storage. Losses in

22 aesthetic values are difficult to quantify. Although foliar symptoms cannot always be classified

23 as damage, their occurrence indicates that phytotoxic concentrations of O_3 are present, and,

24 therefore, studies should be conducted to assess the risk to vegetation.

25   Visible symptoms due to O_3 exposures reduce the market value of certain crops and

26 ornamentals for which leaves are the product, e.g., spinach, petunia, geranium, and poinsettia.

27 The concept of limiting values used to summarize foliar symptoms in the 1978 document (U.S.

28 Environmental Protection Agency, 1978) was also considered valid in the 1986 document (U.S.

29 Environmental Protection Agency, 1986). Jacobson (1977) developed limiting values by

30 assessing the available scientific literature and identifying the lowest exposure

31 concentration/duration reported to cause foliar symptoms in a variety of plant species.

1   A graphical analysis presented in those documents indicated that the limit for reduced plant

2   performance was an exposure to 50 ppb for several hours per day for more than 16 days.

3   Decreasing the exposure period to 10 days increased the concentration required to cause

4   symptoms to 100 ppb; and a short, 6-day exposure further increased the concentration to cause

5   symptoms to 300 ppb. These limiting values established in 1978 were still deemed appropriate

6   in the 1986 and 1996 criteria documents. Such foliar symptoms are caused by O_3 concentrations

7   that occur in the United States as shown in Table 9-13 (as adapted from U.S. Environmental

8   Protection Agency, 1996).

9   The 1986 document emphasized that, although foliar symptoms on vegetation are often an

10   early and obvious manifestation of O_3 exposure, O_3 effects are not limited to foliar symptoms.

11   Other effects include reduced growth of many organs (including roots), changes in crop quality,

12   and alterations in plant susceptibility to biotic stressors and sensitivity to abiotic stressors. The

13   1986 document also emphasized that O_3 exerts phytotoxic effects only if a sufficient amount of

14   O_3 reaches sensitive sites within the leaf (Section 9.3). Ozone injury will not occur if the rate of

15   O_3 uptake is low enough that the plant can detoxify or metabolize O_3 or its metabolites or if the

16   plant is able to repair or compensate for the effects (Tingey and Taylor, Jr., 1982; U.S.

17   Environmental Protection Agency, 1986). Cellular disturbances that are not repaired or

18   compensated for are ultimately expressed as foliar symptoms, reduced root growth, or reduced

19   yield of fruits or seeds.

20   Beginning in the 1986 document and continuing in the 1996 document, OTC studies were

21   reviewed that better quantified the relationship between O_3 exposure and effects on crop species,

22   with a focus on yield loss. These studies can be grouped into two types, depending on the

23   experimental design and statistical methods used to analyze the data: (1) studies that developed

24   predictive equations relating O_3 exposure to plant response, and (2) studies that compared the

25   effects of discrete treatment level(s) to a control. The advantage of the regression approach is

26   that exposure-response models can be used to interpolate results between treatment levels.

27   Discrete treatment experiments were designed to test whether specific O_3 treatments were

28   different from the control rather than to develop exposure-response equations, and the data were

29   analyzed using analyses of variance. When summarizing these studies using discrete treatment

30   levels, the lowest O_3 concentration that significantly reduced yield was determined from

31   analyses done by the original authors. Often, the lowest concentration used in the study was the

Table 9-13. Summary of Ozone Exposure Indices Calculated for 3- or 5-Month Growing Seasons from 1982 to 1991[a]

		3-month growing season (June-August)									
		HDM2[c] ppm		M7 ppm		SUM00 ppm·h		SUM06 ppm·h		SIGMOID ppm·h	
Year	No. of Sites[b]	Mean	CV[d]	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1982	99	0.114	23.7%	0	18.7%	82.9	19.1%	26.8	68.8%	26.3	56.7%
1983	102	0.125	24.9%	0	21.9%	86.1	22.1%	34.5	58.1%	33.0	52.3%
1984	104	0.117	24.6%	0	18.2%	84.1	19.9%	27.7	58.4%	27.4	47.9%
1985	117	0.117	24.6%	0	17.1%	84.6	18.0%	27.4	59.6%	27.4	47.6%
1986	123	0.115	21.8%	0	19.1%	85.3	18.0%	27.7	65.0%	27.7	51.8%
1987	121	0.119	22.9%	0	17.6%	86.9	17.3%	31.2	56.4%	30.4	46.8%
1988	139	0.129	21.3%	0	17.8%	97.6	19.6%	45.2	46.8%	42.9	42.4%
1989	171	0.105	23.1%	0	17.5%	86.4	19.9%	24.8	78.7%	25.8	59.4%
1990	188	0.105	21.6%	0	18.3%	85.7	21.0%	25.8	76.2%	26.6	59.2%
1991	199	0.106	22.0%	0	18.4%	87.7	21.3%	28.3	74.2%	28.9	59.5%
Among Years		0.113	11.1%	0	10.0%	87.0	9.9%	29.5	42.1%	29.4	31.0%

		5-month growing season (May-September)								
		M7 ppm		SUM00 ppm·h		SUM06 ppm·h		SIGMOID ppm·h	
Year	No. of Sites	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).

1   lowest concentration reported to reduce yield; hence, it was not always possible to estimate a no-

2   effect exposure concentration. In general, the data indicated that 100 ppb O_3 (frequently the

3   lowest concentration used in the studies) for a few hours per day for several days to several

4   weeks usually caused significant yield reductions of 10 to 50%.

5   By the time the 1986 document was prepared, much new information concerning the

6   effects of O_3 on the yield of crop plants had become available through EPA's NCLAN research

7   program and through research funded by other agencies. The NCLAN project was initiated by

8   EPA in 1980 primarily to improve estimates of yield loss under field conditions and to estimate

9   the magnitude of crop losses caused by O_3 throughout the United States (Heck et al., 1982,

10   1991). The cultural conditions used in the NCLAN studies approximated typical agronomic

11   practices. The primary objectives were:

12   (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;

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

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

15   Using NCLAN data, the O_3 concentrations predicted to cause 10 or 30% yield loss were

16   estimated using linear or Weibull response functions. The data in Table 9-14 are from the 1996

17   document and were based on yield-response functions for 38 species or cultivars developed from

18   studies using OTCs of the type developed by Heagle et al. (1973) (see Section 9.2). Composite

19   exposure-response functions for both crops and tree seedlings as a function of O_3 exposure

20   expressed as SUM06 are shown in Figure 9-18. Review of these data indicate that 10% yield

21   reductions could be predicted for more than 50% of experimental cases when: (1) 12-h SUM06

22   values exceeded 24.4 ppm·h, (2) SIGMOID values exceeded 21.5 ppm·h, or (3) 7-h seasonal

23   mean concentrations were 50 ppb. The SIGMOID index is very similar to the W126 index (see

24   Section 9.5 for further information about O_3 indices). Much lower values are required for each

25   index to protect 75% of experimental cases (Table 9-15). Grain crops were generally found to

26   be less sensitive than other crops. The data summarized in the 1996 criteria document also

27   indicated that the variation in sensitivity within species may be as great as differences between

28   species.

Table 9-14. 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).
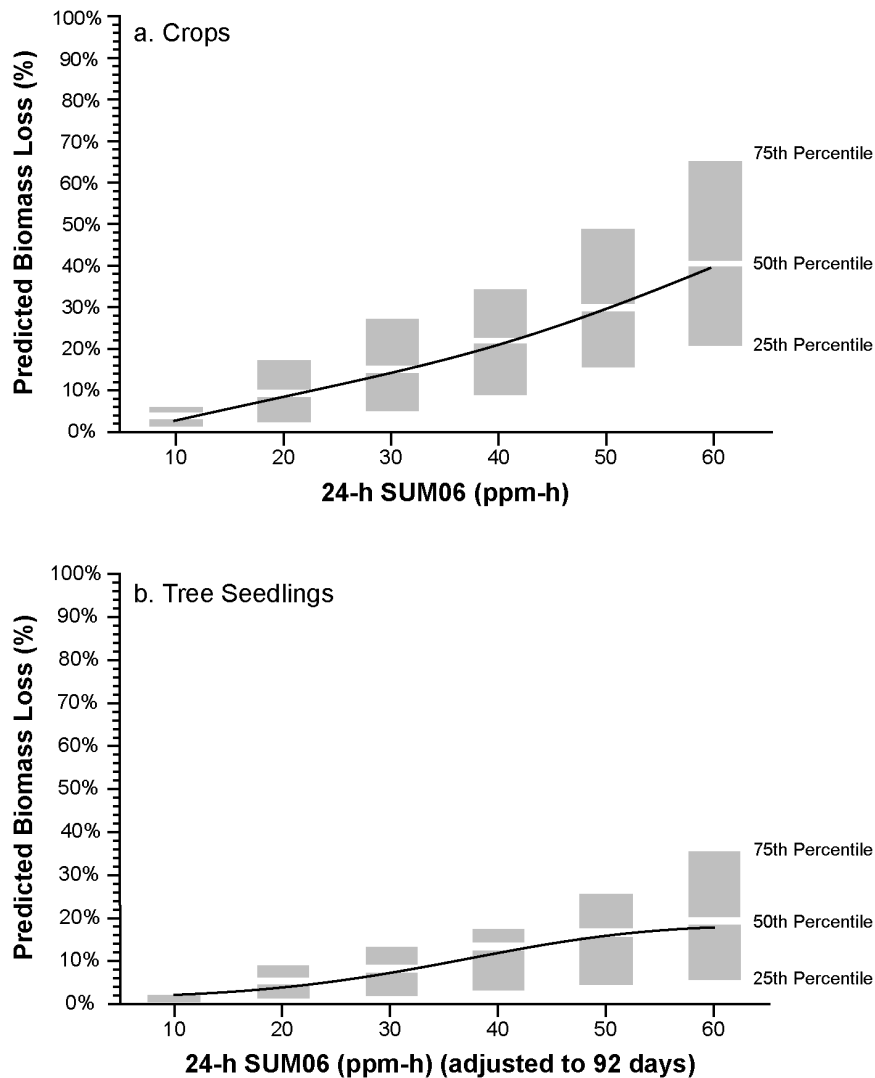
Figure 9-18. **Distribution of biomass loss predictions from Weibull and linear exposure-response models that relate biomass to O₃ exposure. Exposure is characterized with the 24-h SUM06 statistic using data from (a) 31 crop studies from National Crop Loss Assessment Network (NCLAN) and (b) 26 tree seedling studies conducted at U.S. Environmental Protection Agencys' 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).

1   The chemical protectant, ethylene diurea (EDU), was also used to provide estimates of

2   yield loss. The impact of O_3 on yield was determined by comparing the yield data from plots

3   treated with EDU versus untreated plots. Studies indicated that yields were reduced by 18 to

4   41% when daytime ambient O_3 concentrations exceeded 80 ppb for 5 to 18 days over the

5   growing season. For this approach to be credible, the effects of EDU itself on a particular

6   species must be preestablished under conditions without O_3 exposure (Kostka-Rick and

7   Manning, 1992).

8   The 1996 criteria document reviewed several experiments demonstrating that the seedlings

9   of some tree species such as poplars (*Populus*) and black cherry are as sensitive to O_3 as are

10   annual plants, in spite of the fact that trees are longer-lived and generally have lower rates of gas

11   exchange, and, therefore, a lower uptake of O_3. The 1996 document also reviewed data showing

12   that O_3 exposures that occur at present in the United States are sufficient to affect the growth of a

13   number of trees species. For example, exposure-response functions for 51 cases of tree seedling

14   responses to O_3, including 11 species representing deciduous and evergreen growth habits,

15   suggest that a SUM06 exposure for 5 months of 31.5 ppm·h would protect hardwoods from a

16   10% growth loss in 50% of the cases studied (Table 9-15). Similarly, a SUM06 exposure of

17   42.6 ppm·h should provide the same level of protection for evergreen seedlings. However, these

18   results do not take into the account the possibility of effects on growth in subsequent years.

19   Because multiple-year exposures may cause a cumulative effect on the growth of some trees

20   (Simini et al., 1992; Temple et al., 1992), it is likely that a number of species are currently being

21   affected even at ambient exposures (Table 9-14).

22   In 1986, the EPA (U.S. Environmental Protection Agency, 1986) established that 7-h per

23   day growing season mean exposures to O_3 concentrations above 50 ppb were likely to cause

24   measurable yield loss in agricultural crops. At that time, few conclusions could be drawn about

25   the response of deciduous or evergreen trees or shrubs, due to the lack of information about

26   response of such plants to season-long exposures to O_3 concentrations of 40 to 60 ppb and above.

27   However, the 1978 and 1986 criteria documents (U.S. Environmental Protection Agency, 1978,

28   1986) indicated that the limiting value for foliar symptoms on trees and shrubs was 60 to 100

29   ppb for 4 h. From 1986 to 1996, extensive research was conducted, establishing the sensitivity

30   of many tree species. Based on research published since the 1986 criteria document,, a number

31   of conclusions were drawn in 1996 AQCD (U.S. Environmental Protection Agency, 1996):

**Table 9-15. 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[1] = 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 9-15 (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 [XXX] for abbreviations and acronyms.
[b] PRYL = predicted relative yield (biomass) loss

Source: U.S. Environmental Protection Agency (1996), based on Hogsett et al. (1995).

1   (1)   An analysis of 10 years of monitoring data from more than 80 to almost 200 non-urban 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 9-14).

2   (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 9-14) 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   (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   (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 (Table 9-14).

5   (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   (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   (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.

8   The major question to be addressed in the remainder of this section is whether new
9   information supports or alters the 1996 criteria document conclusions summarized above.
10   In particular, this section evaluates whether the response of plants to experimental treatments at
11   or near O$_3$ concentrations characteristic of ambient levels in many areas of the United States
12   (Table 9-14) can be compared to a control or reduced O$_3$ treatment to establish a potential
13   adverse effect. Before evaluating new information from the literature on O$_3$ effects on
14   vegetation, O$_3$ exposure indices used in O$_3$ studies and trends in O$_3$ exposure patterns during the
15   past two decades are briefly reviewed.
16

9.6.3 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 is addressed in Section 9.5, 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 9.3). 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., 1995; Runeckles, 1974; Taylor, Jr. et al., 1982; Tingey and Taylor, Jr., 1982) (see also Section 9.5). 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 9.6.4.5.

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 9.5). 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). For this reason, 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 ozone, 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).

1	Since the 1980s, cumulative indices such as the SUM06, AOT40, or W126 that

2	preferentially weight higher concentrations have been used in conjunction with mean indices for

3	developing exposure-response relationships (Tables 9-15 and 9-16, and Figure 9-1). Such

4	indices are often more suitable than mean values because they are cumulative and because they

5	preferentially weight higher concentrations. Thus, these indices generally provide somewhat

6	better fits to experimental data than do mean indices, especially in retrospective analyses of

7	multiple experiments on multiple species (Lee et al., 1994a, 1994b; Lee and Hogsett, 1999;

8	Tingey et al., 1991). Unfortunately, no single index has been used consistently even in the

9	recent literature, making it difficult to compare results among and between experiments and with

10	ambient exposure data. However, Tables 9-14 and 9-17 provide summaries of ambient exposure

11	data for several indices that can be compared to the experimental results reviewed in the

12	remainder of this section. Of the cumulative indices that preferentially weight higher

13	concentrations, the SUM06 index has been used most commonly in the U.S. literature, and it was

14	selected in a meeting of scientific experts on O_3 effects on vegetation as suitable for a secondary

15	standard to protect vegetation (Heck and Cowling, 1997). However, it should be noted that the

16	W126 index has been selected for use in protecting vegetation in Class 1 areas (Federal Land

17	Managers' Air Quality Related Values Workgroup (FLAG), 2000). Even in recent studies, O_3

18	data are often presented using only a seasonal mean index value, and so mean values are

19	frequently presented in this section. Such reporting of mean indices should not be interpreted as

20	a preference for them, but rather as a limitation in the data reported in the literature. Additional

21	information about O_3 exposure for individual experiments, including the number and type of O_3

22	treatments (addition of a constant concentration of O_3 or an amount proportional to ambient

23	levels), and duration, are reported in Tables 9-16 through 9-19.

24	Since the 1996 document, the use of the AOT40 index has become quite common in

25	Europe for identifying and mapping areas of exceedance, but it has not been used much in the

26	United States. Thus, studies reporting O_3 exposure only as AOT40 values are presented in tables

27	summarizing effects on annual, herbaceous perennial, and woody vegetation. However, such

28	studies are not as commonly cited in the text of this section because AOT40 summary data on O_3

29	exposures in the United States are rarely available. This lack makes it difficult to compare

30	experimentally derived exposure-response data expressed as AOT40 to ambient U.S. O_3

Table 9-16. Summary of Selected Studies of Ozone Effects on Annual Species

Species	Facility	Location	O₃ 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	g=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)
Corn	OTC	Beltsville, MD	CF, +40: 7-h mean = 20, 70	1 years	Grain yield	13	Mulchi et al. (1995) Rudorff et al. (1996c)
Cotton, cv. Deltapine	OTC	Raleigh, NC	CF, 1.5×: 12-h mean = 21, 71	1 years	Seed-cotton weight	22	Heagle et al. (1999)
Cotton, cv. Deltapine	OTC	Raleigh, NC	CF, NF, 1.5×, 12-h mean = 24, **51**, 78	1 years	Seed-cotton weight	21, 49 (NF, 1.5×)	Heagle et al. (1999)
Oat, cv. Vital	OTC	Ostad, Sweden	CF, NF: 7-h mean = 12, **27**	1 years	Grain yield	+2 (n.s.)	Pleijel et al. (1994a)
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)

Table 9-16 (cont'd). Summary of Selected Studies of Ozone Effects on Annual Species

Species	Facility	Location	O₃ Concentration (Units are ppb unless otherwise specified)[1]	Duration	Variable	Response (Decrease from lowest, %)	Reference
Soybean, cvs. Forrest, Essex	OTC	Maryland	CF, +40: 7-h mean = 24 and 24, 63 and 62 for each year	2 years	Seed yield	10, 32 (2 cvs.)	Chernikova et al. (2000)
Soybean, cv. Essex	OTC	Raleigh, NC	CF, NF, 1.5×: 12-h mean = 20, **50**, 79	1 year	Seed yield	16, 37 (NF, 1.5×)	Heagle et al. (1998)
Soybean, cv. Essex	OTC	Raleigh, NC	CF, NF, 1.5×: 12-h mean = 18, **42**, 69	1 year	Seed yield	15, 40 (NF, 1.5×)	Heagle et al. (1998)
Soybean, cv. Holladay	OTC	Raleigh, NC	CF, NF, 1.5×: 12-h mean = 18, **42**, 69	1 year	Seed yield	22, 36 (NF, 1.5×)	Heagle et al. (1998)
Soybean, cv. NK-6955	OTC	Raleigh, NC	CF, NF, 1.5×: 12-h mean = 18, **42**, 69	1 year	Seed yield	+46, +4 (NF, 1.5×)	Heagle et al. (1998)
Soybean, 3 cvs.	OTC	Raleigh, NC	CF, NF, 1.5×: 12-h mean = 14, **36**, 64	3 months	Seed yield	At ambient = +14, 11, 16 for 3 cvs.	Miller et al. (1994)
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 (ns), Forrest = 21	Robinson and Britz (2000)
Timothy	OTC	Sweden	AOT40 = 10, 20, 340; 12-h mean = 20, 152	1 year	Biomass	58	Danielsson et al. (1999)
Watermelon	OTC	Spain	CF (O₃ = 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)

Table 9-16 (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], cv. Minaret	**OTC**	**8 sites in N Europe**	**12-h mean (SD) low = 26.3 (12.2), 12-h mean (SD)-high = 51.4 (18.3)** **AOT40 mean (SD) low = 6.18 (8.54) ppm•h, AOT40 mean (SD) high = 28.23 (23.05) ppm•h**	**13 studies of 1 year each**	**Grain yield**	**13 (n.s.)**	Bender et al. (1999) Hertstein et al. (1999)
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 d/week, 7 weeks	Grain yield	53	Finnan et al. (1996a)
Wheat, cv. promessa	OTC	SE Ireland	CF, +25: 12-h total = 6.2, 33.4 ppm•h	6 h/day, 5 d/week, 7 weeks	Grain yield	+17	Finnan et al. (1996a)
Wheat, cv. promessa	OTC	SE Ireland	CF, +25, +50: 12-h total = 6.7, 34, 34 ppm•h	+25 = 6 h/day, 5 d/week, +50 = 3 h/day, 5 d/week, both 7 weeks	Grain yield	Amb + 25 = 3 (n.s.); Amb + 50 = 17	Finnan et al. (1996a)
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)

Table 9-16 (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, winter, 8 cvs.	OTC	Raleigh, NC	12-h mean = 27, 47, 90	2 months	Grain yield	5 (n.s.)	Heagle et al. (2000)
Wheat, winter, 8 cvs	OTC	Raleigh, NC	12-h mean = 22, 38, 74	2 months	Grain yield	16 (n.s.)	Heagle et al. (2000)
Wheat, cv. Drabant	OTC	Finland	1992: 12-h mean = 14, 30, 61; AOT40 = 16.3, 34.8, 54.6 ppm•h. 1993: 12-h mean = 9, 21, 45; AOT40 = 10.2, 24.8, 40.6 ppm•h	2 years	Grain yield	At highest O_3 = 13 each year	Ojanpera et al. (1998)
Wheat, cv. Riband	Open Air	Northumberland, UK	AOT40 for Mar to Aug 93 = 3.5, 6.2 ppm•h	1 year, overwinter	Grain yield	13	Ollerenshaw and Lyons (1999)

[1] Values for ambient or NF treatments are indicated in bold.
[2] **Bold** indicates that multiple experiments (more than just 2 years at a single site) were included in the analysis.

Table 9-17. Summary of Selected Studies of the Effects of Ozone on Perennial Herbaceous Plants

Species	Facility	Location	O₃ 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 O₃ 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 9-17 (cont'd). Summary of Selected Studies of the Effects of Ozone on Perennial Herbaceous Plants

Species	Facility	Location	O₃ 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)
Lespediza, 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)
Rasberry	OTC	Ontario	1×, +12 +24: Ambient ≤ 4	1 day/week for 7 weeks	Fruit yield	No effect at +12, 52 at +24 in only one of two cultivars	Sullivan et al. (1994)
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 Kingdon	8-h mean = 27, 92; AOT40 for +O₃ = 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	ns 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 9-18. 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 = 7-69; for 1991 = 22-92	98 days	Total biomass	ns at 1×, 29 at 2× for all clones in each year	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, +90 (exposure data not reported)	3 years	Volume (d2*h)	21 at +90	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. (2000a)

Table 9-18 (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. (2001)
Beech, European	0-3 years	OTC	Switzerland	AOT40 for 24 h/days = 4-73 ppm•h	1-3 years	Total biomass	20 at AOT40 for 24 h = 32 ppm•h	Braun and Fluckiger (1995)
Beech, Japanese	4 years	Growth chamber	Japan	CF, +60 ppb for 7 h/day	156 days	Total biomass	19	Yonekura et al. (2001)
Birch, silver	Sapling	FACE	Finland	AOT40 = 1, 15 ppm•h; 7-h mean = 26, 40	5 years	Biomass	34 for root, ns 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 ns at NF+, 22 at NF++; root biomass 30 at NF++	Karlsson et al. (2003)
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)
Cherry, black	2 years	OTC	Norris, TN	CF, 1×, 2×: 7-h mean = 21, **50**, 97	April to August	Biomass	No effect	Samuelson (1994)
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)

Table 9-18 (cont'd). Summary of Selected Studies of Ozone Effects on Deciduous Trees and Shrubs

Species	Age	Facility	Location	O₃ Concentration (Units are ppb unless otherwise specified)[1]	Duration	Variable	Response (Decrease from lowest, %)	Reference
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 = O-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; SUMOO for 3 years = 147, 255 and 507 ppm•h	3 years	Total biomass	n.s.	Samuelson et al. (1996)

Table 9-18 (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)
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 9-18 (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)
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)

[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 9-19. Summary of Selected Studies of Ozone Effects on Evergreen Trees and Shrubs

Species	Age	Facility	Location	O₃ 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, ponderosa	Seedling	OTC	Corvallis, OR	For CF 12-h SUM06 = 0 ppm•h; for +O3 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)
Pine, ponderosa	39 to 45 years	Ambient gradient	CA	24-h mean for 3 weeks late July and early August for 1993 and 1994 = 70-90 ppb	Ambient gradient	Fine and medium root growth	85 at most polluted site.	Grulke et al. (1998)
Pine, ponderosa	Seedling	OTC	CA	CF, 1×, 2×: 24-h mean approx. 20, **60**, 120		Total biomass	n.s.	Takemoto et al. (1997)

Table 9-19 (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, Scots		OTC	Hampshire, UK	NF, NF+: Mean = **17.7**, 44.1; 24-h AOT40 = **1.9**, 59.9 ppm•h	3 years for 62 days	Total biomass	15	Broadmeadow and Jackson (2000)
Pine, Scots	3-6 years	Free air	Finland	Amb, +O_3: AOT40 = **0-1**, 2-13 ppm•h	3 years	Biomass	No effect	Kainulainen et al. (2000)
Pine, Scots	Seedling	OTC	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)
Sequoia, giant	125 years	Branch chamber	California	0.25×, 1×, 2×, 3×: 24-h SUM00 approx. 10, **85**, 180, 560 ppm•h	61 days	Branch growth	No effect	Grulke et al. (1996)
Spruce, Norway	4-7 years	Open air	Finland	Amb, +O_3: AOT40 = **0 1**, 2-13 ppm•h	3 years	Biomass	No effect	Kainulainen et al. (2000)

Table 9-19 (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
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	Seedling	OTC	Sweden	CF, 1×, 1.5×: AOT40 daylight for 4 years = 1, **16**, 79 ppm•h	4 years	Stem volume	No effect in final year	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.

1   exposures. The development of critical levels in Europe has been based primarily on the AOT40

2   index, so this index is discussed in that context.

3   In addition to peak-weighting, there is also evidence that the timing of exposure during

4   plant growth is important. For example, the greatest effects on grain yield are due to exposure

5   during grain filling, rather than earlier or later in the growing season (Lee et al. 1988; Pleijel

6   et al., 1998; Soja et al., 2000; U.S. Environmental Protection Agency, 1996; Younglove et al.,

7   1994). The importance of respite times was also discussed in the previous criteria documents

8   (U.S. EPA, 1978, 1988, 1996) but remains difficult to quantify (Section 9.5). Even when some

9   of these aspects of O_3 exposure can be elucidated, it is difficult to apply this knowledge to

10   developing exposure-response relationships based on data in the scientific literature, because O_3

11   exposure is often reported only in the form of a summary index such as a 12- or 24-h mean,

12   SUM06, or AOT40.

13   Table 9-14 presents summaries of ambient O_3 exposure patterns in the United States for

14   1982 to 1981 for several indices including the 7-h mean and SUM06. More recent summaries

15   for the entire United States for these indices are not available, but Table 9-15 summarizes more

16   recent data for the central and eastern United States. As shown in Table 9-15, from 1989 to

17   1995, mean 12-h 3-month SUM06 values (in ppm-h) at 41 rural sites in the Clean Air Status and

18   Trends Network were 31.5 for the Midwest, 18.9 for the Upper Midwest, 33.2 for the Northeast,

19   13.2 for the Upper Northeast (NH, ME), 34.5 for the South-Central, and 19.2 for the Southern

20   Peripheral subregions (Baumgardner and Edgerton, 1998). These results are important because

21   these sites were selected to represent rural areas, while many other monitoring sites represent

22   urban or suburban areas. For these same subregions, W126 values ranged from 12.8 to

23   25.6 ppm-h. From 1989 to 1995, O_3 concentrations decreased about 5% for daily and 7% for

24   weekly values for most of these sites, after adjusting for meteorological conditions (Holland

25   et al., 1999). These trends were statistically significant at about 50% of the sites ($p \leq 0.05$).

26   However, because the trend analysis was intended to examine the efficacy of O_3 emissions

27   controls, the trends were adjusted for meteorological conditions. Thus, they do not reflect the

28   actual trends in O_3 exposure over time.

29

Table 9-20. Ethylene Diurea Effects on Vegetation Responses to Ozone

Species	Description	EDU application	Ozone exposure	Effects of EDU	Reference
Bean, cv. Lit	10-cm pots in OTCs in Germany	Soil drench 200 mL of 150 ppm solution per plant every 14 days	CF, NF, CF-1×, CF-2×: mean = 1, 14, 15, 32 ppb	O_3 reduced pod, shoot, and root mass. EDU increased root, leaf, and shoot mass, but a significant interaction with O_3 occurred only for root weight.	Brunschon-Harti et al. (1995)
Bean, cv. BBL-290	2 expts in 5.5 L pots in OTCs with 4 O_3 treatments	Soil drench every 14 days, in expt 1 = 0.14, 28, 56, 120 mg/L potting medium; expt 2 = 0, 8, 16, 32 mg/L	2 expts with CF, NF and 2 constant additions of O_3. 7-h mean O_3 (ppb) for Expt 1 = 34, 70, 95, 121; Expt 2 = 19, 42. 74, 106	Visible injury and reduced total biomass or yield, even in CF treatment. Within an O_3 treatment, sometimes increased yield (Expt 2 only).	Miller et al. (1994)
Bean	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)
Clover, subterranean	Plants in 10-cm pots at 4 rural sites in the Netherlands for 3 years	100 ml of 150 ppm solution as soil drench every 14 days for 2 months	AOT40 = 0-0.56 ppm-h for 4-week periods	Injury, but not leaf biomass was affected by EDU and O_3 exposure.	Tonneijck and Van Dijk (2002)

Table 9-20 (cont'd). Ethylene Diurea Effects on Vegetation Responses to Ozone

Species	Description	EDU application	Ozone exposure	Effects of EDU	Reference
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)

9.6.4   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 below in Section 9.6.4.6.

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. (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 9.2 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 recent studies of individual species to those reviewed in the 1996 AQCD.

9.6.4.1   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 recently by (Black et al. 2000}. As discussed in Section 9.4, 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

1   growth and, because planting or germination dates vary throughout large regions even for a

2   single species, no single phenological weighting scheme can appropriately and practically

3   represent all vegetation in all locations in the United States. For natural populations, reductions

4   in seed yield might be particularly important if subsequent seedling establishment is

5   compromised by O_3.

6   Green beans (cv. Pros) were grown in pots in OTCs in the Netherlands for 62 days and

7   exposed to 6 treatments consisting of constant O_3 additions to charcoal filtered (CF) chambers

8   (see Section 9.2) for 9 h/day (Tonneijck and Van Dijk, 1998). Bean yield response to O_3 was

9   nonlinear, with an apparent threshold near the CF30 (charcoal filtered with a constant addition of

10   30 ppb O_3) treatment with a 9-h mean O_3 concentration of 28 ppb and an AOT40 value of

11   0.1 ppm·h). Yield was reduced by 29% at a 9-h mean value of 44 ppb corresponding to an

12   AOT40 value of 3.6 ppm·h (Table 9-17). Beans were grown in pots in OTCs for 3 months with

13   the following O_3 treatments: CF, nonfiltered (NF), CF with O_3 added up to ambient, and CF

14   with 2×-ambient O_3 (Brunschon-Harti et al., 1995). Ozone reduced pod mass by 56% with a

15   mean concentration of 32 ppb in the 2× ambient treatment as compared with 1 ppb in the CF

16   treatment (daily averaging time not reported). A second treatment factor in this experiment was

17   addition of EDU, as discussed below under the heading "Studies Using Ethylene Diurea as a

18   Protectant". These yield reductions are greater than those previously reported in four similar

19   studies summarized in the 1996 AQCD (Table 5-25 of U.S. Environmental Protection Agency,

20   1996). Greater sensitivity in the more recent experiments may be due to cultivar differences or

21   other differences in experimental protocols.

22   In a study with OTCs on silty loam soil in Beltsville, MD, corn yield was reduced by 13%

23   with exposure to a 7-h mean concentration of 70 ppb O_3 compared to a CF treatment with a 7-h

24   mean concentration of 20 ppb (Mulchi et al., 1995; Rudorff et al., 1996a; Rudorff et al., 1996c).

25   In this study, different amounts of O_3 were added above ambient levels for 5 days follows: 20,

26   30, 40, 50, 60 ppb, except that O_3 was not added to exceed a total concentration of 120 ppb

27   (Rudorff et al., 1996c, 1996a).

28   In two studies conducted in Raleigh, NC, cotton (cv. Deltapine 51) was grown in pots and

29   exposed to CF and 1.5× (nonfiltered, see Section 9.2) O_3 in one year, and CF, NF, and

30   1.5×-ambient O_3 in the second year, with ambient and elevated CO_2 concentrations (Heagle

31   et al., 1999; Table 9-17). In the first year, yield decreased by 22% with 1.5×-ambient O_3 (12-h

1   mean value of 71 ppb). In the second year, yield decreased by 21% and 49% with exposure to

2   ambient or 1.5× ambient O_3 (12-h mean values of 51 and 78 ppb, Table 9-17). Increased CO_2

3   levels prevented or reduced this yield suppression (Heagle et al., 1999). These yield reductions

4   are similar to those reported previously in four similar studies summarized in the 1996 AQCD

5   (Table 5-25 of U.S. Environmental Protection Agency, 1996).

6   In a study of oats in OTCs in southern Sweden, exposure to ambient (NF) O_3 did not affect

7   grain yield (Pleijel et al., 1994a). Ambient O_3 concentration expressed as a 7-h mean was

8   27 ppb, with only 1 h greater than 80 ppb and none above 90 ppb.

9   The interactive effects of elevated O_3 and CO_2 additions on potato yield (cv Bintje) were

10   studied in OTCs at 6 sites in northern Europe as part of the CHIP (Changing Climate and

11   Potential Impacts on Potato Yield and Quality) program (Craigon et al., 2002). Ozone was

12   added to a target daily average value of 60 ppb, and AOT40 values across all years and

13   experiments ranged from ~6 to 27 ppm·h. The O_3 treatment reduced total tuber yield an average

14   of 4.8% with elevated O_3 treatment across all experiments (Craigon et al., 2002). This total

15   effect was statistically significant even though the effects of individual experiments generally

16   were not (Craigon et al., 2002), due to the increased power of the pooled analysis. Several

17   publications report other aspects of the CHIP experiments or present results of individual

18   experiments (De Temmerman et al., 2002a, 2002b; Donnelly et al., 2001a, 2001b; Fangmeier

19   et al., 2002; Finnan et al., 2002; Hacour et al., 2002; Lawson et al., 2002; Pleijel et al., 2002;

20   Vandermeiren et al., 2002; Vorne et al., 2002).

21   The effect of an intermittent constant addition of O_3 using a free air exposure system in

22   Northumberland, UK was investigated with the oilseed rape cultivar "Eurol" (Ollerenshaw et al.,

23   1999). Ozone was added for 6 h/day for 17 days. The ambient treatment had a mean value of

24   30 ppb and the O_3 addition treatment had a mean of 77 ppb. After overwintering, O_3 was added

25   for 32 days for 7 h/day between May and June (mean values of 31 and 80 ppb). Yield was

26   reduced by 14% despite the lack of any foliar symptoms.

27   Field fumigation chambers ventilated with fans on both ends were used to assess effects of

28   five O_3 treatments on rice over 3 years in Japan (Kobayashi et al., 1994; 1995). All O_3

29   treatments used CF air, and O_3 was added to the 0.5, 1.0, 1.5, 2.0, or 2.75× ambient

30   concentration for 7 h/day. Based on a linear regression for the 3 years, yield decreased by 3 to

31   10% at a 7-h mean concentration of 40 ppb (Table 9-17). This decrease is greater than that

1 found for rice in earlier studies in California (Kats et al., 1985), although whether this difference

2 is due to differences in cultivars, experimental treatment, or environmental factors cannot be

3 determined.

4   During 3 years in Beltsville, MD, the soybean cultivars Essex and Forrest were exposed to

5 CF air and NF air in OTCs (Chernikova et al., 2000; Robinson and Britz, 2000) with O_3 added as

6 described for experiments with corn and wheat at Beltsville (Mulchi et al., 1995; Rudorff et al.,

7 1996c). During 1994 and 1995, as previously found for these cultivars, Essex was less sensitive

8 than Forrest, with yield decreases of 10% (n.s.: p > 0.1) compared to 32% for Forrest (p < 0.01)

9 (Chernikova et al., 2000). There was no evidence of water stress in this experiment. In 1997,

10 the two O_3 treatments were CF (7-h mean = 24 ppb) and NF with a constant addition of O_3 (7-h

11 mean = 58 ppb) (Robinson and Britz, 2000). The yield of Essex was not significantly affected,

12 while the yield of Forrest was decreased by 21% (Table 9-17).

13   In a study in Raleigh, NC the soybean cultivar Essex was grown in pots and exposed to CF

14 and 1.5× ambient O_3 concentrations during three growing seasons (Fiscus et al., 1997). Over the

15 3 years, exposure to an average 12-h mean O_3 concentration of 82 ppb reduced soybean yield by

16 41% (Table 9-17). In similar studies also in Raleigh, NC, Essex was exposed to CF, NF, and

17 1.5× ambient O_3 for two seasons (Heagle et al., 1998). Yield decreased by 16% and 15% in the

18 2 years by ambient O_3 (12-h mean values of 50 and 42 ppb), and decreased by 37 and 40% with

19 exposure to 1.5× ambient O_3 (12-h mean values of 79 and 69 ppb, Table 9-17). In this same

20 experiment in the second year, similar yield reductions were observed for the cultivar Holladay,

21 while the growth of cultivar NK-6955 was increased substantially by ambient O_3 exposure.

22 All three cultivars were grown in the same chambers in this experiment, and the authors

23 suggested that NK-6955 plants may have shaded the other cultivars to some extent.

24   In a 2-year study using OTCs in Raleigh, NC, the soybean cultivars Coker 6955, Essex,

25 and S53-34' were exposed CF, NF, and 1.5× ambient O_3 treatments (Miller et al., 1994).

26 Seasonal mean 12-h O_3 concentrations ranged from 14 to 83 ppb. As compared to the CF

27 treatment, ambient O_3 exposure (NF treatment) reduced seed yield by 11 to 18% except for

28 Coker 6955 in the first year (1989) which showed a yield increase of 14%. The 1.5× ambient O_3

29 treatment reduced yield by 32 to 56% in all cultivars in both years. In a similar subsequent

30 experiment with the cultivar Essex, exposure to a 12-h mean ambient O_3 concentration of 50 ppb

1   reduced yield by 11%, while exposure to 79 ppb reduced yield by 22% (Table 9-17) (Miller

2   et al., 1998).

3   These yield reductions for soybean are generally similar to those reported previously in

4   13 similar studies summarized in the 1996 AQCD (Table 5-23 of U.S. Environmental Protection

5   Agency, 1996).

6   A reanalysis of 7 years of data from OTC experiments with wheat in Ostad, Sweden

7   showed that relative yield linearly decreased with increasing O_3, with a maximum yield loss of

8   23% at an AOT40 value of 15 ppm·h (Danielsson et al., 2003). A very similar response was

9   found using the flux (stomatal conductance) model of Emberson et al. (2000b), and a similar

10   amount of the variance was explained by the flux model (for AOT40 model, $r^2 = 0.34$ and for the

11   Emberson flux model, $r^2 = 0.39$). A modified flux model developed and calibrated for this site

12   also had a similar linear response equation, but explained much more of the variance ($r^2 = 0.90$).

13   During the 1990s, a major European research program investigated the combined effects of

14   CO_2, O_3, and other physiological stresses on wheat (Bender et al., 1999; Hertstein et al., 1996,

15   1999; Jäger et al., 1999). The European Stress Physiology and Climate Experiment ("ESPACE-

16   wheat") program included 13 experiments in OTCs at eight sites in northern Europe over 3

17   years. Low- and high-O_3 exposures in these experiments had the following values: 12-h mean

18   (SD) low = 26.3 ppb (12.2), 12-h mean (SD) high = 51.37 (18.3) ppb, AOT40 mean (SD) low =

19   6.2 (8,5) ppm-h, AOT40 mean (SD) high = 28.3 (23.0) ppm-h, as calculated from data presented

20   in Table 3 of Hertstein et al. (1999). An analysis of all 13 experiments showed that high O_3 at

21   ambient CO_2 reduced yield by 13% on average (Bender et al., 1999). However, this reduction

22   was not statistically significant based on an ANOVA, and the authors concluded that the wheat

23   cultivar Minaret may be relatively tolerant to O_3 (Bender et al., 1999). Results of some

24   individual studies within this program have been reported previously (Donnelly et al., 1999;

25   Fangmeier et al., 1996, 1999; Mulholland et al., 1997, 1998b, 1998a; Pleijel et al., 2000b).

26   In a study with OTCs on silty loam soil in Beltsville, MD, wheat yield was reduced by

27   20% on average over 2 years with 7-h mean concentrations of 61 and 65 ppb O_3 compared with

28   CF treatment with a 7-h mean concentration of 20 ppb (Mulchi et al., 1995; 1996a; Rudorff

29   et al., 1996c). In the above study, different amounts of O_3 were added above ambient (levels for

30   5 days) as follows: 20, 30, 40, 50, 60 ppb, except that O_3 was not added to exceed a total

31   concentration of 120 ppb (Rudorff et al., 1996c). Wheat grown in pots in OTCs was exposed to

1   elevated O_3 and water stress in Germany, and yield was decreased by 35% in the 2×-ambient

2   treatment with a 7-h mean O_3 concentration of 71 ppb statistically significant effects were not

3   seen in the 1×-ambient treatment (Fangmeier et al., 1994). In two studies conducted in Raleigh,

4   NC, soft red winter wheat was grown in pots and exposed to CF, NF, and 1.5×-ambient O_3, with

5   ambient and elevated CO_2 concentrations (Table 9-17) (Heagle et al., 2000). In the first

6   experiment, eight cultivars were exposed to 12-h mean O_3 concentrations of 27, 47, and 90 ppb,

7   and in the second experiment two of these cultivars were exposed to 22, 38, and 74 ppb. There

8   was a trend toward decreased yield in both experiments, but these trends were not statistically

9   significant. The wheat cultivar Drabant was exposed to CF, NF, and a constant addition of

10   35 ppb during 1992 and 1993 using the Heagle-type OTCs (Heagle et al., 1973) in Finland

11   (Ojanpera et al., 1998). The following 12-h mean O_3 exposures were observed in 1992: 14, 30,

12   61 ppb. In 1993, the values were 9, 21, and 45 ppb, (see Table 9-17 for AOT40 values and other

13   information). Yield was reduced 13% in each year by the added O_3 treatment.

14   The effect of an intermittent constant addition of O_3 using a free air exposure system was

15   investigated with the winter wheat cultivar Riband in Northumberland, UK (Ollerenshaw and

16   Lyons, 1999). Ozone exposures expressed as AOT40 values for September and October 1992

17   were 0.14 and 3.5 ppm·h; while for April to August 1993, values were 3.5 and 6.2 ppm·h. Yield

18   was reduced by 13%.

19   These results provide an additional line of evidence supporting the OTC-studies that

20   demonstrated yield reductions in wheat due to O_3 exposures that occur in the United States.

21   These yield reductions for wheat are generally similar to those reported previously in

22   22 comparable studies summarized in the 1996 AQCD (Table 5-25 of U.S. Environmental

23   Protection Agency, 1996).

24

25   **9.6.4.2   Effects on Plant Quality**

26   In addition to reductions in biomass or crop yield, O_3 may also reduce the quality or

27   nutritive value of annual species. Many studies have shown effects of O_3 on various measures of

28   plant organs that affect quality, with most studies focusing on characteristics important for food

29   or fodder (U.S. Environmental Protection Agency, 1996).

30   The effect of a continuous intermittent addition of O_3 using a free air exposure system in

31   Northumberland, UK was investigated with the oilseed rape cultivar Eurol as discussed above

1   (Ollerenshaw et al., 1999). Ozone exposures expressed as AOT40 values for August to October

2   1991 were 0.2 and 3.8 ppm·h and for June 1992 were 0.7 and 8.1 ppm·h. Yield quality measured

3   as crude protein and oil content was decreased significantly. Because the price of the product is

4   reduced in direct proportion to the oil content, such a decrease represents a substantial loss to

5   growers (Ollerenshaw et al., 1999).

6   Two wheat cultivars, Massey and Saluda, were each grown for one year each in Beltsville,

7   MD (Table 9-17) and exposed to either CF or an addition of 40 ppb for 7 h/day for 5 days/week

8   (Mulchi et al., 1995, 1996a; Rudorff et al., 1996c). Milling and baking quality scores and flour

9   protein were not significantly affected by elevated O_3 exposure, but the softness equivalent was

10   increased slightly (2.4%) in both experiments (Rudorff et al., 1996b). The authors concluded

11   that these changes, along with other slight changes due to an increased CO_2 treatment, suggested

12   that O_3 and CO_2 had only minor effects on wheat grain quality. In wheat grown in Sweden, the

13   harvest index was significantly decreased and the protein content increased due to exposure to a

14   12-h mean of 48 ppb (Gelang et al., 2000). In an analysis of 16 experiments conducted with

15   spring wheat and either O_3 or CO_2 exposures in four Nordic countries, a negative linear

16   relationship was found between grain yield and grain protein content ($y = -0.38 \times + 138.6$,

17   expressed as percentages of the NF treatment (Pleijel et al., 1999a).

18   For three soybean cultivars grown in Raleigh, NC, O_3 significantly decreased oleic acid

19   content, although the authors stated that the reduction was not large enough to be economically

20   important (Heagle et al., 1998).

21   In a UK study, potato exposed during 1998 to an AOT40 value of 12.5 ppm·h in OTCs

22   (in Nottingham) resulted in the paste from tubers being more viscous (Donnelly et al., 2001b).

23   In this study, an AOT40 exposure of 27.11 ppm·h in 1999 caused starch granules to be less

24   resistant to swelling, and total glycoalkaloid content was increased due to an increase in a-

25   solanine (Donnelly et al., 2001b). Such increases in glycoalkaloid content have been observed

26   previously in potato (Pell and Pearson, 1984) and may be important, because glycoalkaloids

27   cause bitter flavors and, at higher concentrations, cause toxicity. The authors indicated that

28   levels found in this study approached those that may cause bitterness, but not those of concern

29   for toxicity (Donnelly et al., 2001b).

30   In the CHIP program the effects of O_3 were studied using OTCs at six sites in northern

31   Europe, and yield decreases were observed as described above. The reducing sugar and starch

1   content of tubers decreased linearly due to O_3 exposure, while the ascorbic acid concentration

2   increased linearly (Vorne et al., 2002). Compared to the CF treatment, exposure to an AOT40

3   value of 14 ppm·h decreased starch concentrations by 2%, decreased reducing sugar

4   concentration by 30%, and increased ascorbic acid concentration by 20%. While the changes in

5   reducing sugars and ascorbic acid increase tuber quality, the reduction in starch concentration

6   decreases tuber quality.

7   In two 1-year studies using OTCs in commercial fields in Spain, the soluble solids content

8   of watermelon was decreased 4 to 8% due to seasonal O_3 exposures as follows: AOT40 = 5.96

9   ppm·h and SUM06 = 0.295 ppm·h in one year; and AOT40 = 18.9, SUM06 = 4.95 in the second

10 year (Gimeno et al., 1999).

11

12 **9.6.4.3   Effects on Foliar Symptoms**

13   For most annual crop species, the most important effects of O_3 are on yield of the

14 commercially important part of the crop, expressed as the mass of the harvested portion.

15 However, for some crops, foliar symptoms are important if they reduce the marketability of the

16 crop. This is why efforts have been made to identify O_3 exposures associated with foliar

17 symptoms. In Europe, Level I critical levels have been determined for such effects based on

18 observations from experiments conducted in 15 countries under the auspices of the United

19 Nations Economic Commission for Europe International Cooperative Programme on effects of

20 air pollution and other stresses on crops and non-woody plants (UN/ECE ICP-Vegetation;

21 formerly ICP-Crops), as well as on observations of symptoms in commercial fields from 1993 to

22 1996 (Benton et al., 1995, 2000). Because the occurrence of symptoms increased with greater

23 humidity, these levels took into account the vapor pressure deficit (VPD). Two short-term

24 critical levels were derived from 1995 data: an AOT40 value of 0.2 ppm·h over 5 days when

25 mean VPD is below 1.5 KPa (0930 - 1630 h), and a value of 0.5 ppm·h when the mean VPD is

26 above 1.5 Kpa (Benton et al., 1996). The 1996 data supported the critical levels in 83% of

27 observations, although symptoms occurred on three occasions when the AOT40 was less than

28 0.05 ppm·h and the VPD was very low — less than 0.6 Kpa. The authors concluded that these

29 critical levels are a good indicator of the likelihood of foliar symptoms, but that further

30 refinement may be required, such as including factors that modify O_3 uptake by stomata.

1   In a study in Germany, 25 native herbaceous species were exposed to several square-wave

2   O_3 exposures in CF OTCs (Bergmann et al., 1999). Six of the 25 species showed O_3-specific

3   symptoms, and five species responded to single-day peaks of a single day. The most sensitive

4   species exhibiting O_3-specific symptoms were *Cirsium arvense* and *Sonchus asper*, which

5   responded to AOT40 values < 1.5 ppm·h (Bergmann et al., 1999).

6   In the United States, attention has been paid to foliar symptoms on annual plants in

7   previous criteria documents, and most recent literature has focused on other topics. However, a

8   study of O_3 effects was undertaken from 1990 to 1993 in Acadia National Park in Maine, which

9   experiences elevated O_3 exposures due to transport from urban areas located upwind (Kohut

10   et al., 2000). Because this study examined both herbaceous perennial species and woody

11   perennial species, the results are discussed in the perennial section below. Other studies and

12   biomonitoring programs in other U.S. National Parks and over larger areas are oriented primarily

13   towards forested ecosystems, so such studies are also discussed in the perennial subsection

14   below as well as in Section 9.7.

15

16   **9.6.4.4   Other Effects**

17   In addition to the effects seen on individual species grown in monocultures, effects may

18   occur due to competition when species are grown in mixtures, as reviewed by Davison and

19   Barnes (1998). Such effects have been investigated primarily in species grown for forage, which

20   often include perennial species; therefore, this topic is discussed in the herbaceous perennial

21   section, Section 9.6.5.1. Other stresses may interact with O_3, including pathogens and pests as

22   well as environmental conditions such as drought. These issues are discussed in Section 9.4.

23   Effects on reproduction are discussed briefly below.

24   Several studies during recent decades have demonstrated O_3 effects on different stages of

25   reproduction. Effects of O_3 have been observed on pollen germination, pollen tube growth,

26   fertilization, and abortion of reproductive structures as reviewed by Black et al. (2000). This

27   issue is not addressed here because reproductive effects will culminate for seed-bearing plants in

28   seed production, and the substantial body of evidence relating O_3 exposure and reduced seed

29   production is discussed above. However, one example of a native species will be presented

30   because of its implications for extrapolating exposure-response data to noncommercial species.

31   Spreading dogbane has been identified as a useful species for O_3 biomonitoring because of

1   O_3-induced diagnostic symptoms (Kohut et al., 2000). A study in Massachusetts found that

2   exposure to O_3 in NF OTCs or ambient plots for 103 days produced significantly fewer flowers,

3   and also that fewer of these flowers survived to produce mature fruits (Bergweiler and Manning,

4   1999). Because foliar symptoms were not common, the authors concluded they are not required

5   for effects on reproduction to occur. Genotoxic effects and effects on population genetics are

6   discussed in Section 9.4.

7

8   **9.6.4.5   Scaling Experimental Data to Field Conditions**

9   Substantial effort has been invested in the design of OTCs for assessing the effects of air

10   pollutants on vegetation under near-ambient conditions. The design, construction, and

11   performance of many types of chambers has been reviewed extensively (Hogsett et al., 1987a,

12   1987b). Despite such design efforts, the influence of experimental chambers on exposure-

13   response functions has been debated for many years (e.g., Manning and Krupa, 1992) because

14   several factors differ between OTC studies and actual fields. This issue is addressed in Section

15   9.2, and only a few comments about the implications of chamber artifacts for interpreting

16   exposure-response relationships are presented here.

17   While it is clear that chambers can alter some aspects of plant growth, the more important

18   issue is whether they alter the response of plants to O_3. A review of such chamber studies done

19   in California found that plants responded similarly to O_3 whether OTCs, closed-top chambers, or

20   air exclusion systems were used; differences were found for fewer than 10% of growth

21   parameters (Olszyk et al., 1986). In a different review of literature about Heagle-type OTCs

22   (Heagle et al., 1988), the authors concluded that "Although chamber effects on yield are

23   common, there are no results showing that this will result in a changed yield response to O_3."

24   A more recent study of chamber effects examined the responses of tolerant and sensitive white

25   clover clones to ambient O_3 in greenhouse, open-top, and ambient plots (Heagle et al., 1996).

26   For individual harvests, O_3 reduced the forage weight of the sensitive clone 7 to 23% more in the

27   greenhouse than in OTCs. However, the response in OTCs was the same as in ambient plots.

28   Several studies have shown very similar yield response to O_3 for plants grown in pots or in the

29   ground, suggesting that even such a significant change in environment does not alter the

30   proportional response to O_3, at least as long as the plants are well watered (Heagle, 1979; Heagle

31   et al., 1983).

1   Most experiments investigating the O_3 effects on annual vegetation provide adequate water

2   to avoid substantive drought stress. Because drought stress has generally been shown to reduce

3   the effect of O_3 on annual vegetation, such experiments may tend to overestimate O_3 effects on

4   crops and especially on unmanaged or seminatural vegetation.

5   As mentioned above, the use of O_3 flux rather than exposure is theoretically more realistic,

6   and such an approach would also address the vertical gradient issue (Section 9.2). Recently, a

7   number of investigators have suggested that modeling O_3 flux can improve estimates of O_3

8   effects on vegetation. Models of O_3 flux can reduce the variation in the response to O_3 that is

9   sometimes observed between years in an experiment (Emberson et al., 2000a, 2000b; Fuhrer

10   et al., 1992; Grünhage et al., 1993; Grünhage and Haenel, 1997; Pleijel et al., 2000a). In a study

11   of O_3 deposition to an oat crop in OTCs, O_3 flux in the chamber was estimated to be up to twice

12   that in an adjacent field based on a K-theory approach and measurements of stomatal

13   conductance and environmental conditions (Pleijel et al., 1994b). These measurements were

14   made for 2 hours on 5 days when the canopy was physiologically active and wind speeds were

15   moderate. However, the O_3 flux in a chamber without plants was nearly as high as that in the

16   open field. The authors conclude that O_3 uptake in the chamber was between 100 and 200% of

17   that in the field. These models of flux have a sound biological and meteorological basis and are

18   useful for interpreting experimental data. Flux models have been successfully applied at

19   intensive study sites with detailed site-specific data on stomatal conductance and

20   micrometeorological conditions (e.g., Grünhage et al., 1993b, 1994; Fredericksen et al., 1996).

21   Yet even at a single well-studied site, different methods can provide different estimates of O_3

22   flux. For example, at a site in a vineyard in California, an evapotranspiration-based method

23   overestimated the O_3 flux as compared to an eddy covariance approach by 20 to 26% (Massman

24   and Grantz, 1995). At a site in a nearby cotton field the evapotranspiration-based approach

25   overestimated the eddy-covariance method by 8 to 38%.

26   Interest has been increasing in recent years, particularly in Europe, in using mathematically

27   tractable flux models for O_3 assessments at the regional and national scale (Emberson et al.,

28   2000a, 2000b). However, methods for scaling site-specific models of O_3 flux to large areas

29   remains an active and challenging area of research (Massman et al., 1994; Massman and Ham,

30   1994; Wesely and Hicks, 2000). Reducing uncertainties in flux estimates for areas with diverse

31   surface or terrain conditions to within ±50% requires "very careful application of dry deposition

1   models, some model development, and support by experimental observations" (Wesely and

2   Hicks, 2000). As an example, the annual average deposition velocity of O_3 among three nearby

3   sites in similar vegetation was found to vary by ±10%, presumably due to terrain (Brook et al.,

4   1997). Moreover, the authors stated that the actual variation was even greater, because stomatal

5   uptake was unrealistically assumed to be the same among all sites, and flux is strongly

6   influenced by stomatal conductance (Brook et al., 1997). Stomatal conductance is affected by

7   factors such as temperature, vapor pressure deficit, plant water status, and temperature. It is a

8   challenging task to obtain such data for regional, national, or continental flux modeling (Pleijel,

9   1998). This topic is addressed further in Section 9.5.

10   If a flux approach is not used, another issue in scaling from experimental data to national

11   assessments is the selection of an O_3-exposure index. As discussed in Section 9.5, there is no

12   evidence to support a single value for defining a cutoff value for calculating a peak-weighted O_3

13   index. In Europe, it has become common practice to use a cutoff value of 40 ppb, as in the

14   AOT40 index; while in the United States, a cutoff value of 60 ppb has been used, as in the

15   SUM06 index. The W126 index has been promoted, because it has a continuous weighting

16   function without an arbitrary cutoff value; but few exposure-response studies have used this

17   index to date. There is evidence from some European studies that a cutoff value either lower

18   (Pleijel et al., 1997) or higher (Finnan et al., 1996, 1997) may provide a better statistical fit to

19   experimental data. However, the choice of the type of weighting depends on the type of

20   response model that is fit. In an evaluation of numerous indices fit to seven studies of the

21   response of spring wheat to O_3 in OTCs in Northern Europe, different types of indices performed

22   better for linear response models than for Wiebull response models (Finnan et al., 1997). In this

23   study, cumulative indices that give greater weight to higher O_3 concentrations performed best;

24   and, while the AOT40 was not the best index, it did perform well, and slightly better than the

25   SUM06 in conjunction with a linear model (Finnan et al., 1997). Although this section is

26   focused on annual plants, the issue of cutoff values applies to perennial species as well;

27   therefore, two examples of tree species will be discussed here. Aspen seedlings were exposed to

28   O_3 in controlled environment chambers for 4 weeks, and a significantly greater degree of foliar

29   symptoms occurred with a pattern of variable peak exposures compared to a constant peak

30   exposure with the same total SUM06 exposure (Yun and Laurence, 1999b). However, a field

31   experiment in large OTCs in Ithaca, NY with these two clones exposed to ambient, 1.7×,- and

1   3×-ambient O_3 concentrations (SUM06 = 1, 20, and 62 ppb) found SUM06 to be highly

2   correlated with foliar symptoms and biomass effects (Yun and Laurence, 1999a). When silver

3   birch was exposed to different patterns of O_3 with the same AOT40 value in growth chambers,

4   high peak concentrations and exposure shape were found to be important for symptom

5   production (Oksanen and Holopainen, 2001). However, growth reductions were best predicted

6   by total cumulative exposure.

7   An investigation of different exposure indices was conducted using data from experiments

8   conducted during 1993 at eight sites in the eastern United States in which O_3 sensitive and

9   O_3-tolerant white clover genotypes were grown using methods developed by Heagle et al.

10 (1994). A statistical approach based on profile likelihoods was used to estimate parameters in

11 generalized exposure indices similar to the SUM06 and AOT40 indices (Blankenship and

12 Stefanski, 2001). The results showed that for the SUMX family of indices, where X is a cutoff

13 value, hourly O_3 concentrations over ~71 ppb contribute the most to yield prediction. For the

14 AOTX family of indices, the parameter was 54.4. These values are similar to those used in the

15 SUM06 and AOT40 indices already in use. Furthermore, investigation of weighting for time of

16 day confirmed the importance of the mid-afternoon hours for this data set, unlike the results

17 found for wheat in Sweden (Danielsson et al., 2003; Pleijel et al., 2000a).

18

19 **9.6.4.6   European Critical Levels**

20   During the late 1980s and 1990s, substantial effort was expended in Europe to quantify the

21 effects of O_3 on crops and trees. A focus for this research effort was to develop and refine

22 "critical levels" for O_3 under the auspices of the United Nations Economic Commission for

23 Europe (UNECE) Convention on Long-Range Transboundary Air Pollution (Sanders et al.,

24 1995). A critical level was defined as "the concentration of pollutant in the atmosphere above

25 which direct adverse effects on receptors , such as plants, ecosystems or materials, may occur

26 according to present knowledge" (United Nations Economic Commission for Europe, 1988).

27 Areas where the levels were exceeded were identified to plan abatement strategies. Critical

28 levels are not air quality standards as are used in the United States for criteria pollutants, nor are

29 they legally enforced. However, they have been used successfully for planning reductions in

30 sulfur and nitrogen pollution. Critical levels for O_3 are intended to prevent long-term deleterious

31 effects on the most sensitive plant species under the most sensitive environmental conditions, but

1   not to quantify O_3 effects. The nature of the "significant harmful effects" is not specified in the

2   original definition, which allows different levels to be defined for different effects, such as foliar

3   symptoms or reduction in crop yield. Different levels also have been set for crops, forests and

4   seminatural vegetation. The caveat, "according to present knowledge" is important, because

5   critical levels are revised periodically as new scientific information becomes available.

6   Because these critical levels were designed for use in regional modeling and mapping, they

7   were required to be of a simple form (Sanders et al., 1995). During the early 1990s, the AOT40

8   index was selected as the form of the critical level, and Level I critical levels were determined

9   for arable crops and forestry. The AOT40 index is defined as the sum of the difference between

10 the hourly concentration (in ppb) and 40 ppb when the concentration exceeds 40 ppb for the

11 hours when global radiation exceeds 50 W m^{-2} (Fuhrer et al., 1997). In regression analysis of

12 15 OTC studies of spring wheat, including one study from the United States and 14 from

13 locations ranging from southern Sweden to Switzerland, an AOT40 value of 5.7 ppm·h was

14 found to correspond to a 10% yield loss, and a value of 2.8 ppm·h corresponded to a 5% yield

15 loss (Fuhrer et al., 1997). Because a 4 to 5% yield loss could be detected with a confidence level

16 of 99%, a critical level of 3 ppm·h was selected an AOT40 value of in 1996 (Kärenlampi and

17 Skärby, 1996). This critical level is defined for a 3-month period calculated for daylight hours.

18 It was suggested that a 5-year average should be used to assess the exceedance of this critical

19 level, because this value was associated with a small yield decrement (Fuhrer et al., 1997). This

20 value is currently used for all crops, because it is the best-supported value and because the

21 limited data from other crop species do not provide strong evidence that a more stringent value is

22 required (Fuhrer et al., 1997).

23   The Level I critical levels were successfully used in the 1990s to map areas of exceedance,

24 but the research led to the conclusion that simple, exposure-based levels lead to overestimation

25 of the effects in some regions and underestimation in others. The main problem is that other

26 environmental factors (e.g., vapor pressure deficit, water stress, temperature, light) alter O_3

27 uptake and its effects. Therefore, the decision was made to work towards a flux-based approach,

28 aiming to be able to model O_3 flux-effect relationships for the three vegetation types (i.e., crops,

29 forests, seminatural vegetation). Progress has been made in modelling flux under experimental

30 conditions (Danielsson et al., 2003; Grünhage et al., 2001; Grünhage and Jäger, 2003; Pleijel

1   et al., 2000a), but because the UNECE meeting in November 2002 concluded that more research

2   is needed, the subject is to be reassessed in 2 to 5 years' time.

3   There has been criticism that the Level I critical level for crops overestimates O_3 effects in

4   the Mediterranean countries, because it was developed based on studies in Northern Europe

5   (De Santis, 1999, 2000). However, there is evidence of substantial crop loss due to O_3 in some

6   southern European counties, such as the Po valley in Northern Italy (Fumagalli et al., 2001).

7   In these studies Heagle type OTCs were used. Losses in NF chambers as compared to CF

8   chambers over several years at two sites ranged from 11.2 to 22.8% for barley and wheat, from

9   0.3 to 31.5% for other crop species, and from 4.1 to 19.8% for forage species (Fumagalli et al.,

10   2001). Surprisingly, the least effect was observed for soybean, despite AOT40 values of 9.32

11   ppm·h, 3× the Level I critical level. Similarly, a review of studies in Northern Italy found that

12   ambient O_3 episodes have been reported to cause foliar symptoms on 24 agricultural and

13   horticultural crops in commercial fields (Fumagalli et al., 2001). Ambient O_3 has also been

14   reported to cause yield losses in several crop species, although no data on O_3 exposure were

15   presented by the reviewers (Fumagalli et al., 2001).

16   The Level I approach has also been criticized for focusing only on a single annual species

17   (wheat) and a single woody perennial species (beech). However, this species focus is

18   appropriate, because the goal was to determine an exposure-response relationship for a sensitive

19   species based on available data. In support of standards in Germany, an effort was made to

20   combine data from different species, and consisted of a meta-analysis of studies conducted in

21   both closed chambers and OTCs (Grünhage et al., 2001). In this study, experiments published

22   between 1989 and 1999 were analyzed if they met the following conditions: (1) a significant O_3

23   effect was determined (2) exposure conditions were well defined, (3) foliar symptoms, growth,

24   or yield was measured, and (4) plant species were relevant to Europe (Grünhage et al., 2001).

25   Despite the focus on European species, many of the species studies also occur in the United

26   States. Separate regressions for herbaceous plants and for tree species were created as a function

27   of duration of exposure at a given level of O_3 exposure at the top of the plant canopy. These

28   regression equations, with confidence limits and with correction for the vertical gradient in O_3

29   from the top of the quasi-laminar boundary layer, can be used to define whether effects are

30   unlikely (below the lower confidence limit), probable (between the confidence limits), or highly

31   likely (above the upper confidence limit) to occur near a given O_3-monitoring station.

1   A further concern about the Level 1 approach is that foliar symptoms, rather than biomass,

2   may be an important endpoint, because foliar symptoms may be more sensitive than is biomass

3   increment (VanderHeyden et al., 2001). In an OTC study in southern Switzerland, it was shown

4   that a number of tree species show foliar symptoms at AOT40 values lower than the Level 1

5   value of 10 ppm·h (VanderHeyden et al., 2001).

6

7   **9.6.4.7   Summary of Effects on Short-Lived Species**

8   For annual vegetation, the data summarized in Table 9-17 show a range of growth and

9   yield responses both within species and among species. Nearly all of these data were derived

10   from studies in OTCs, with only two studies using open-air systems in the UK (Ollerenshaw

11   et al., 1999; Ollerenshaw and Lyons, 1999). It is difficult to compare studies that report O_3

12   exposure in different indices such as AOT40, SUM06, or 7-h or 12-h mean values. However,

13   when such comparisons can be made, the results of this recent body of research confirm earlier

14   results summarized in the 1995 AQCD (U.S. Environmental Protection Agency, 1996).

15   A summary of earlier literature concluded that a 7-h, 3-month mean of 49 ppb corresponding to a

16   SUM06 exposure of 24.4 ppm·h would cause 10% loss in 50% of 49 experimental cases (Tingey

17   et al., 1991). A similar study using a 24-h, rather than 7-h, averaging period found that a

18   SUM06 exposure of 26.4 ppb would cause 10% loss in 50% of 54 experimental cases (Lee et al.,

19   1994a, 1994b). Recent data summarized in Table 9-17 support this conclusion. These values

20   represent ambient exposure patterns that occur in some years over large portions of the United

21   States. Some annual species such as soybean are more sensitive, and greater losses may be

22   expected (Table 9-17). Thus, the recent scientific literature supports the conclusions of the 1996

23   AQCD that ambient O_3 concentrations are reducing the yield of major crops in the United States.

24   Much research in Europe has used the AOT40 exposure statistic, and substantial effort has

25   gone into developing Level-1 values for vegetation. Based on regression analysis of 15 OTC

26   studies of spring wheat, including one study from the United States and 14 from locations

27   ranging from southern Sweden to Switzerland, an AOT40 value of 5.7 ppm·h was found to

28   correspond to a 10% yield loss, and a value of 2.8 ppm·h corresponded to a 5% yield loss

29   (Fuhrer et al., 1997). Because a 4 to 5% decrease could be detected with a confidence level of

30   99%, a critical level of an AOT40 value of 3 ppm·h was selected in 1996 (Kärenlampi and

31   Skärby, 1996).

1	In addition to reductions in crop yield, O_3 may also reduce the quality or nutritive value of
2	annual species. Many studies have shown effects of O_3 on various measures of plant organs that
3	affect quality, with most studies focusing on characteristics important for food or fodder. These
4	studies indicate that there may be economically important effects of ambient O_3 on the quality of
5	crop and forage species. Previous criteria documents have concluded that foliar symptoms on
6	marketable portions of crops and ornamental plants can occur with seasonal 7-h mean O_3
7	exposures of 40 to 100 ppb (U.S. Environmental Protection Agency, 1978, 1986, 1996). The
8	recent scientific literature does not refute this conclusion.
9	The use of OTCs may reverse the usual vertical gradient in O_3 that occurs within a few
10	meters above the ground surface (Section 9.2). Such a reversal suggests that OTC studies may
11	overestimate, to some degree, the effects of an O_3 concentration measured several meters above
12	the ground. However such considerations do not invalidate the conclusion of the 1996 AQCD
13	(U.S. Environmental Protection Agency, 1996) that ambient (Table 9-14, Table 9-21) O_3
14	concentrations are sufficient to reduce the yield of major crops in the United States.
15	
16	

Table 9-21. Ozone Exposures at 35 Rural Sites in the Clean Air Status and Trends Network in the Central and Eastern United States From 1989 to 1995

Subregion	SUM06 12-h, 3-Month Mean	UM06 12-h, 3-Month SD	W126 3-Month Mean	W126 3-Month SD	Max. 8h > 80 ppb(n) Mean	Max. 8h > 80 ppb(n) SD
Midwest	31.5	10.2	25.1	7.7	13.8	10.6
Upper Midwest	18.9	8.5	16.0	5.9	5.6	5.6
Northeast	33.2	11.9	26.6	9.5	15.8	12.2
Upper Northeast	13.2	8.6	12.8	6.5	3.3	5.5
South Central	34.5	16.6	25.6	11.5	7.1	10.0
Southern Periphery	19.2	7.6	15.2	5.4	1.9	1.6

Units for SUM06 and W126 are ppm-h.
Source: Baumgardner and Edgerton (1998).

9.6.5   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 recent 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 9.6.5.1 reviews such studies, with an emphasis on studies using OTCs.

9.6.5.1   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, which 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 (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 9-18). 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

1   shorter total O_3 exposure time as well as to the lower O_3-exposure concentrations during the

2   weeks immediately preceding harvest. These results are important, because this is an

3   economically important species and because previous studies have focused on grass species that

4   use the C_3, rather than C_4, metabolic pathway.

5   An investigation of the use of different O_3 indices and averaging times on the correlation

6   with growth effects was undertaken with the NC clover system (Heagle et al., 1995). For 2 years

7   of data at six sites in Massachusetts, Oregon, North Carolina, California (2 sites), and Virginia,

8   averaging time was found to be more important than the choice of the type of index including

9   mean, SUM06, and AOT40 (Heagle and Stefanski, 2000). The best correlation between O_3

10 exposure and the ratio of sensitive to tolerant clover types was found for the 6-h period from

11 1000 to 1600 h. For this period, very similar r^2 values (0.91 to 0.94) were found for SUM06,

12 W126, and AOT40 (Heagle and Stefanski, 2000). For the above indices, a linear relationship

13 was found, with no effect in Corvallis, OR with exposure to a SUM06 value of 10.2 ppm·h and a

14 ratio of 0.53 (sensitive/tolerant) at San Bernardino with a SUM06 exposure of 39.4 ppm·h

15 (Heagle and Stefanski, 2000).

16   In a study of the biomass ratio of O_3-sensitive versus O_3-insensitive clover at 14 sites in

17 Europe during 1996 to 1998, a model that was developed using artificial neural network (ANN,

18 see Section 9.2) techniques had r^2 values for the training data of 0.84 and for unseen validation

19 data of 0.71 (Mills et al., 2000). The predictive factors in the model were AOT40, 24-h mean

20 O_3, daylight mean temperature, and 24-h mean temperature. This model was selected after a

21 thorough investigation of a number of models using many more or fewer parameters using both

22 ANN and multiple linear regression techniques. This model predicted that a 5% reduction in

23 biomass ratio was associated with AOT40 values in the range 0.9 to 1.7 ppm·h accumulated over

24 28 days, with plants being most sensitive under conditions of low NO_x, moderate temperature,

25 and high 24-h mean O_3 concentration.

26   Two experiments in Ontario investigated effects of square-wave additions of O_3 for 7 h on

27 1 day/week for 7 weeks on the growth and fruit production of two cultivars of primocane (first-

28 year fruiting) raspberry plants (Sullivan et al., 1994). Plants were grown in OTCs and exposed

29 to ambient O_3 (mean values of $\leq$ 4 ppb). In the both experiments, additions of 12 ppb O_3 on

30 1 day/week for 7 weeks did not significantly reduce fruit yield. In the second experiment,

1   additions of 24 ppb significantly reduced the fruit yield 52% below that of the ambient treatment

2   in one cultivar (Heritage), but not in another (Redwing).

3   Exposure to a square-wave 8-h mean O_3 concentration of 92 ppb for 62 days in an

4   experiment in OTCs the UK did not significantly reduce the total yield of strawberry fruits, but

5   did decrease the average size of the fruits by 14% (Drogoudi and Ashmore, 2000). This

6   contrasts with an increase in total yield (fruit weight) found in a previous study in California

7   (Takemoto et al., 1988).

8   When timothy was exposed in OTCs in Sweden to NF, CF, and CF+O_3 treatments, there

9   was no effect of a 12-h mean O_3 exposure of 68 ppb (NF treatment), but a 12-h mean exposure

10   of 152 ppb decreased yield by 58% (Danielsson et al., 1999). A similar lack of effect of

11   exposure to a 12-h mean O_3 exposure of 62 ppb was found in a previous study in the United

12   States (Kohut et al., 1988).

13   Although most investigations of O_3-response relationships focus on growth or yield of

14   marketable portions of plants, some studies also investigate effects on plant quality. In the study

15   of bahia grass in Alabama discussed above, in addition to the effects on yield, there were

16   significant effects on quality for ruminant nutrition (Muntifering et al., 2000). Concentrations of

17   neutral detergent fiber (NDF) were higher in primary-growth and regrowth forages from the

18   early-season planting when exposed to 2×-ambient O_3 than when exposed to the NF treatment.

19   The concentration of acid detergent fiber was higher in the 2×-ambient treatment than in NF

20   treatment regrowth, whereas acid detergent lignin concentration was higher in 2×-ambient than

21   in NF primary-growth forage. Crude protein concentrations were lower in CF-exposed than in

22   NF-exposed regrowth forage from the early planting and in CF- than in NF-exposed primary-

23   growth forage from the initial harvest of the late-season planting. No differences were observed

24   among treatments in concentrations of total phenolics in primary-growth or regrowth forages

25   from either planting, although concentrations of total phenolics tended to be higher in

26   CF-exposed than in NF-exposed primary-growth forage from the late-season planting. The

27   authors concluded that the alterations in quality of primary-growth and vegetative regrowth

28   forages were of sufficient magnitude to have nutritional and possibly economic implications to

29   their use for ruminant animal feed.

30   *Sericea lespedeza* and little bluestem were exposed to CF, NF, and 2×-ambient O_3 in OTCs

31   in Alabama for 10 weeks (Powell et al., 2003). Ozone treatments expressed as 12-h mean

1   concentrations were 23, 40, and 83 ppb and expressed as seasonal SUM06 values were 0.2, 9.1,

2   and 61.0 ppm·h. Although there were few statistically significant effects of O_3 on yield (the

3   yield of only the 2×-ambient compared to NF for *Sericea lespedeza* in the last of six harvests),

4   plant quality as feed for ruminants was reduced. The nutritive quality of *Sericea lespedeza* was

5   decreased by 7% and that of little bluestem by 2% as a result of increased cell wall constituents

6   and decreased in vitro digestibility.

7   For some annual species, particularly crops, the endpoint for an assessment of the risk of

8   O_3 exposure can be defined as yield or growth; e.g., production of grain. For plants grown in

9   mixtures such as hayfields, and natural or seminatural grasslands (including native

10   nonagricultural species), endpoints other than production of biomass may be important. Such

11   endpoints include biodiversity or species composition and measures of plant quality such as total

12   protein, and effects may result from competitive interactions among plants in mixed-species

13   communities. Most of the available data on non-crop herbaceous species are for grasslands.

14   In a study of two perennial grasses (bent grass and red fescue) and two forbs (white clover,

15   Germander speedwell) grown in pots in OTCs, O_3 effects differed among species and cutting

16   treatments (Ashmore and Ainsworth, 1995) (see also Table 9-18). Fescue biomass increased

17   with higher O_3 treatments both in pots that were not cut during the growing season (mid-June to

18   mid-September), and those that were cut every two weeks. However, bent grass biomass

19   decreased with higher O_3 exposure in the uncut treatment and increased in the cut treatment.

20   White clover and Germander speedwell biomass decreased substantially with higher O_3 exposure

21   with and without cutting, with greater decreases in the cut treatment. The authors cautioned that

22   the experiment did not replicate field circumstances. The plants were all cut to 1 cm above the

23   ground, which does not simulate grazing, and there may have been effects due to growing the

24   plants in pots. However, two key results of this study likely apply to mixtures of species

25   growing in hay or forage fields or seminatural and natural communities. First, O_3 exposure

26   increased the growth of O_3-tolerant species while exacerbating the growth decrease of O_3

27   sensitive species. Second, the total biomass of the mixed-species community was unaffected by

28   O_3 exposure due to the differential effects on O_3-sensitive and O_3-tolerant species.

29   In a 2-year study using OTCs placed over managed pasture in Switzerland, the above-

30   ground biomass of clover (red and white) was reduced linearly in response to increased O_3

31   exposure (Table 9-18) (Fuhrer et al., 1994). Exposure to a 12-h mean concentration of 39 ppb

1   O$_3$ reduced biomass by 24% as compared to the CF treatment with a 12-h mean concentration of

2   21 ppb O$_3$. There was a trend toward increased above-ground biomass of grasses (primarily

3   orchard grass), but this trend was not statistically significant. As often found in other studies of

4   mixtures of species, by O$_3$ exposure did not significantly affect total above-ground biomass O$_3$

5   exposure.

6   A field-grown grass/clover mixture was exposed to CF, NF, and two O$_3$ addition treatments

7   for two growing seasons in OTCs in southern Sweden (Pleijel et al., 1996). The mixture

8   consisted of 15% (by seed weight) red clover cv. Fanny, 60% timothy cv. Alexander, and 25%

9   fescue cv. Svalofs Sena.. Ozone concentrations expressed as AOT40 ranged from 0 to

10   approximately 47 ppm·h and expressed as 7-h mean from 11 to 62 ppb. Over this range, a slight,

11   but statistically significant, linear decrease of 4% in total above-ground biomass was seen

12   growth over six harvests. No significant decrease was seen in the proportion of clover, and the

13   authors ascribed this lack of effect to the relatively higher O$_3$ sensitivity of timothy and lower

14   sensitivity of this clover cultivar as compared to previously published results for other

15   grass/clover mixtures (e.g., Fuhrer et al., 1994).

16   A mixture of species in an old farm field in Alabama was exposed to O$_3$ for two growing

17   seasons in large OTCs 4.8 m high, and 4.5 m diameter); and a similar lack of effect of O$_3$ was

18   found on total plant community growth measured as both canopy cover and vertical canopy

19   density (Barbo et al., 1998). Of the 40 species in the plots, O$_3$ effects were examined only on the

20   most common species: blackberry, broomsedge bluestem, bahia grass, *Panicum* spp., and

21   winged sumac (second year only). Of these species, a 2×-ambient O$_3$ treatment increased the

22   percent canopy cover of blackberry over 2 years by 124%, while that of winged sumac was

23   decreased by 95% (Table 9-18). Blackberry showed no significant effect on biomass, but ripe

24   fruit mass was decreased by 28% (Chappelka, 2002). However, there was a significant chamber

25   effect for this latter response. Biomass was not reported for other species in this study due to a

26   hurricane. Effects on loblolly pine grown in this experiment are discussed subsequently in

27   Section 9.6.5.5.

28   In summary, results of studies on perennial herbaceous species conducted since the 1996

29   criteria document was prepared are presented in Table 9-18. As for single-season agricultural

30   crops, yields of multiple-year forage crops are reduced at O$_3$ exposures that occur in some years

31   over large areas of the United States (Table 9-14, Table 9-21). This result confirms that reported

1   in the 1996 AQCD (U.S. Environmental Protection Agency, 1996). When species are grown in

2   mixtures, O_3 exposure can increase the growth of O_3-tolerant species while exacerbating the

3   growth decrease of O_3-sensitive species (e.g., Ashmore and Ainsworth, 1995; Fuhrer et al.,

4   1994). Because of this competitive interaction, the total growth of the mixed-species community

5   may not be affected by O_3 exposure (Ashmore and Ainsworth, 1995; Barbo et al., 1998; Fuhrer

6   et al., 1994). However, in some cases mixtures of grasses and clover species have shown

7   significant decreases in total biomass growth in response to O_3 exposure in studies in the United

8   States (Heagle et al., 1989; Kohut et al., 1988) and in Sweden (Pleijel et al., 1996). In Europe,

9   a provisional critical level for perennials of an AOT40 value of 7 ppm·h over 6 months has been

10   proposed to protect sensitive plant species from the adverse effects of O_3.

11

12   **9.6.5.2   Deciduous Woody Species**

13   It is extremely difficult and costly to study entire mature trees under controlled conditions

14   such as those in OTCs, with the possible exception of some species managed for fruit or nut

15   production. For this reason, the great majority of investigations have been of seedlings in

16   growth chambers, greenhouses, or OTCs. A few investigations have been carried out on

17   saplings or more mature trees using free air exposure systems (Haeberle et al., 1999; Isebrands

18   et al., 2000, 2001; Werner and Fabian, 2002). Exposure-response functions based on 28

19   experimental cases of seedling response to O_3 suggest that a SUM06 exposure for 3 months of

20   31.5 ppm·h would protect hardwoods from a 10% growth loss in 50% of the cases (Table 9-19).

21   However, there is a substantial range in sensitivity among species. A risk analysis was

22   undertaken to predict tree biomass growth reductions due to O_3 based on exposure-response

23   equations for seedlings of individual species combined with the species' spatial distribution

24   across the eastern United States and interpolated O_3 exposure expressed as SUM06 (Hogsett

25   et al., 1997). The growth of sensitive species such as aspen and black cherry was predicted to be

26   reduced by at least 20% across 50% of their ranges in a high O_3 year and approximately 10% in a

27   lower-than-average O_3 year (Hogsett et al., 1997).

28   A few investigations reported since the last criteria document was prepared have examined

29   saplings or mature trees, notably of oak species in the southern Appalachian Mountains and pine

30   species in California. Most of these studies have been of natural (uncontrolled) O_3 exposures.

31   Additional studies have examined foliar symptoms on mature trees, and in recent years such

1   surveys have become more common and with greater attention to the standardization of methods

2   and the use of reliable indicator species (Campbell et al., 2000; Smith et al., 2003). Previous

3   criteria documents have noted the difficulty in relating foliar symptoms to effects on individual

4   tree growth, stand growth, or ecosystem characteristics (U.S. Environmental Protection Agency,

5   1996). This difficulty still remains to the present day.

6   Some investigators have suggested that a comprehensive risk assessment of the effects of

7   O_3 on mature tree species might best be accomplished by extrapolating measured effects of O_3

8   on seedlings to effects on forests using models based on tree physiology and forest stand

9   dynamics (Fuhrer et al., 1997; Hogsett et al., 1997; Chappelka, 1998; Laurence et al., 2000,

10   2001). Several such efforts are discussed in Sections 9.4 and 9.7.

11   In this subsection, emphasis will be placed on experimental evidence of O_3 effects on the

12   growth of woody species under controlled conditions with some information from observational

13   studies under ambient conditions in forests. Experimental results are summarized for deciduous

14   species in Table 9-20; and the species are discussed below in the order in which they appear in

15   this table.

16   A series of studies out in Michigan and Wisconsin during the 1990s on clones of trembling

17   aspen previously demonstrated that they differ in their O_3 sensitivity (Coleman et al., 1995b,

18   1995a, 1996; Dickson et al., 2001; Isebrands et al., 2000, 2001; Karnosky et al., 1996, 1998,

19   1999; King et al., 2001). Several of those studies were undertaken with plants in pots or in the

20   ground in OTCs and additional studies were undertaken at three sites selected to differ primarily

21   in O_3 exposure (Karnosky et al., 1999). An ongoing study was undertaken using a free air

22   carbon dioxide and O_3 enrichment (FACE) facility in Rhinelander, WI (Isebrands et al., 2000,

23   2001). These studies showed that O_3-symptom expression was generally similar in OTCs and

24   FACE and gradient sites, supporting the previously observed variation among aspen clones

25   (Karnosky et al., 1999). In the Michigan OTC studies, O_3 decreased total plant biomass by an

26   average of 18% in each of 2 years for three clones classified as high, intermediate and low in O_3

27   tolerance (clones 216, 271 and 259; Karnosky et al. [1996]). However, in the first experiment,

28   an additional clone known to be O_3 insensitive showed no response (clone 253). In these studies,

29   plants were grown in pots and exposed to CF, 1×-ambient, and 2×-ambient O_3 treatments in two

30   separate experiments of 98 days each (additional treatments of 0.5×-ambient and 1.5×-ambient

31   were used in the first experiment only (Karnosky et al., 1996). Ozone concentrations expressed

1   as 3-month, 7-h mean values for 1990 ranged from 7 to 69 ppb for the CF to 2×-ambient

2   treatments; while for 1991, values ranged from 22 to 92 ppb.

3   The FACE study evaluated the effects of 3 years of exposure to combinations of elevated

4   CO_2 and O_3 on growth responses in mixture of five trembling aspen clones (Isebrands et al.,

5   2000, 2001). Height, diameter, and stem volume (diameter^2 × height) were decreased by

6   elevated O_3. On average for all clones, stem volume was decreased by 20% over the 3 years in

7   the elevated O_3 treatment as compared with the 1×-ambient treatment. However, one clone

8   showed increased growth in response to O_3. Ozone concentrations were not reported. This

9   FACE facility study is important, because it confirmed responses reported previously with these

10 clones grown in pots or soil in OTCs, without the alterations of microclimate induced by

11 chambers. Currently, this is the only U.S. study using this technology to have examined the

12 effects of O_3 under these conditions. This study is also significant, because the elevated

13 O_3-exposure pattern used was intended to reproduce the 6-year average pattern from Washtenaw

14 County, Michigan (Karnosky et al., 1999).

15   Rooted cuttings of two aspen clones from Acadia National Park in Maine were exposed to

16 1×-ambient, 1.7×-ambient, and 3×-ambient O_3 concentrations in large OTCs in Ithaca, NY for

17 much of one growing season (15 June to 15 September) (Yun and Laurence, 1999a). Both

18 circular (4.7 m diameter, 3.7 m height) and rectangular (7.4 m × 2.75 m × 3.7 m height)

19 chambers were used (Mandl et al., 1989). Exposure to 1.7×-ambient O_3 (SUM06 = 20 ppm·h,

20 9-h mean = 74 ppb) reduced shoot growth by 14 and 25% compared to ambient O_3 for the two

21 clones (Yun and Laurence, 1999a). Total dry weight was reduced by 55 and 35% in the two

22 clones by the 3×-ambient treatment (SUM06 = 62 ppm·h, 9-h mean = 124 ppb) compared to the

23 ambient O_3 treatment.

24   When black poplar cuttings in OTCs in Belgium were exposed to 8-h mean O_3

25 concentrations of 5, 29, and 33 ppb, diameter growth decreased by 29% in the highest O_3

26 treatment, but height growth was unaffected (Bortier et al., 2000b). A 2-month study of hybrid

27 poplar (*Populus tremuloides* × *P. tremul*a) in a free air exposure system in Finland with 7-h

28 mean O_3 concentrations of 30 and 38 ppb found a 6% decrease in height with no effect on

29 biomass (Oksanen et al., 2001). Eastern cottonwood cuttings in pots buried in the ground with

30 drip irrigation were exposed to ambient O_3 at several sites in and near New York City in three

31 2-month experiments during three summers (Gregg et al., 2003). Ozone concentrations were

1	lower at urban sites than at rural sites within 100 km of the urban sites. Total biomass growth
2	was greater in urban than rural sites, with a strong linear decrease in biomass with increasing O_3
3	across all sites and years ($r^2 = 93$). Total biomass decreased 33% with 12-h mean O_3 levels of
4	38 ppb compared to 23 ppb. Multiple regression analysis showed no significant temperature
5	effect on biomass; therefore, the authors suggested that O_3 exposures was the most likely
6	explanation for the reduced biomass in rural areas. The overall growth reductions and the
7	variation among genotypes seen on all of the above aspen and poplar studies is similar to those
8	previously reported in three OTCs studies summarized in the previous criteria document
9	(Table 9-26 of U.S. Environmental Protection Agency, 1996).
10	Black cherry seedlings grown in pots were exposed in OTCs in the Great Smoky Mountain
11	National Park in Tennessee to O_3 treatments ranging from CF to 2×-ambient in two experiments
12	during 1989 and 1992 (Neufeld et al., 1995). Ozone exposure, expressed as SUM06, ranged
13	from 0 to 40.6 ppm·h in 1989, and from 0 to 53.7 ppm·h in 1992. Corresponding AOT40 values
14	were 0.0 to 28.3 ppm·h in 1989, and 0 to 40.4 ppm·h in 1992. In 1989, total biomass was
15	decreased in the 1.5×-ambient treatment by 18% and in the 2×-ambient treatment by 38%.
16	In 1992, total biomass was decreased in the 1.5×-ambient treatment by 27%, and in the
17	2×-ambient treatment by 59%. In this study, SUM06 and AOT40 provided better fits than did
18	SUM00 with Weibull regression analyses to log-transformed biomass data. Although a Weibull
19	model was used, responses to O_3 expressed as SUM06 and AOT40 appeared to be linear. The O_3
20	exposures in the 1.5×-ambient and 2×-ambient treatments were reported to be similar to those
21	for a site near Charlotte, NC in a high-O_3 year (1988). In a 2-year experiment in OTCs in Ohio,
22	seedlings of black cherry, sugar maple, and yellow poplar were exposed to O_3 treatments with
23	SUM00 values ranging from 16 to 107 ppm·h in 1990 and 31 to 197 ppm·h in 1991 (Rebbeck,
24	1996). After two seasons of exposure, only black cherry showed a growth decrease: total
25	biomass was reduced by 32% in the 2×-ambient O_3 treatment compared to the CF treatment; root
26	biomass was decreased by 39%. These results contrast with those of a previous study with black
27	cherry seedlings in which significant biomass reductions with exposures up to 2×-ambient were
28	not observed (7-h mean = 21 to 97 ppb), perhaps because of the small sample size (3 seedlings
29	per chamber (Samuelson, 1994) in the earlier study.
30	A multiyear study of effects of O_3 on both seedling and mature (30-year-old) red oak trees
31	was conducted in Norris, TN in large OTCs with three replicates per O_3 treatment. Trees were

1   exposed for 3 years to CF, 1×-ambient, and 2×-ambient treatments, with the following

2   O_3 exposures: SUM06 for 3 years = 0, 29, 326 ppm·h; SUM00 for 3 years = 147, 255, and

3   507 ppm·h. The net photosynthetic rate in mature trees was reduced by 25% in the ambient

4   treatment and by 50% in the 2×-ambient treatment (Hanson et al., 1994; Samuelson and

5   Edwards, 1993; Wullschleger et al., 1996). Despite these large decreases, no significant effects

6   on stem increment at the base, stem increment in the canopy, or leaf mass were observed for the

7   mature trees (Samuelson et al., 1996). Similarly, seedling biomass was not significantly reduced

8   by O_3 exposure. The difficulty in replicating experiments with mature trees makes it difficult to

9   detect changes in growth or biomass. However, the mean values of the stem increment at the

10   base and within the canopy in the ambient treatment were larger than those in the CF treatment,

11   although those in the 2×-ambient treatment were lower. Therefore this study of mature trees

12   does not provide evidence that these ambient concentrations reduced aboveground tree growth,

13   even after 4-years exposure.

14   Sugar maple seedlings were exposed for 3 years to ambient, 1.7×-ambient, and 3×-ambient

15   O_3 treatments at both high-light (35% of ambient) and low-light levels (15% of ambient) (Topa

16   et al., 2001). This experiment was conducted in large OTCs near Ithaca, NY. Over the 3 years,

17   O_3 exposures expressed as SUM00 for the three treatments were 88, 126, and 185 ppm·h. After

18   3 years, total seedling biomass in the 3×-ambient treatment was reduced by 64% and 41% in the

19   low- and high-light treatments, respectively (compared to the 1×-ambient treatment). The larger

20   reduction of biomass under low-light conditions suggests that seedlings growing under closed

21   canopies may be substantially more sensitive to O_3 than are seedlings exposed to higher-light

22   levels in gaps or clearings. These results differ from other studies in which seedling biomass

23   was unaffected by exposure to SUM00 values of 304 ppm·h over 2 years (Rebbeck, 1996) or

24   591 ppm·h over 3 years (daytime mean of 40.7) (Laurence et al., 1996). However the latter two

25   studies used much higher light levels, which may have reduced the O_3 effect, based on the results

26   of Topa et al. (2001).

27   Although most studies demonstrate that O_3 decreases biomass growth, occasional results

28   indicate that O_3 can increase growth of some portions of woody perennials. When Casselman

29   plum trees near Fresno, CA were exposed to O_3 in large, rectangular OTCs to three O_3 treatments

30   (CH, 1×-ambient, and an above-ambient O_3 treatment) for 4 years (12-h mean = 31, 48, 91

31   ppm·h), stem increment increased 14% in the highest O_3 treatment compared to the CH

1   treatment; and this effect was statistically significant (Retzlaff et al., 1997). However, fruit yield

2   decreased in this treatment by 42% and also decreased by 16% in the 1×-ambient-O_3 treatment.

3   Root growth was not measured in this study. Hence, the increase in stem diameter may have

4   been at the expense of other organs. However, in a fifth year, all plants were exposed to

5   1×-ambient O_3, and there were no differences in fruit yield, suggesting that trees were able to

6   recover to some extent from the effects of O_3 exposure in prior years.

7   When yellow poplar seedlings were exposed to O_3 concentrations up to SUM00 values of

8   107 ppm·h in one year and 197 ppm·h in a second year, no effects on biomass were observed

9   (Rebbeck, 1996).

10   Many studies have demonstrated that root growth is more sensitive to O_3 exposure than is

11   stem growth. For example, in a study with black cherry seedlings exposed in OTCs in

12   Tennessee in 1989, root biomass in a 2×-ambient treatment was decreased by 42%, while stem

13   biomass was decreased by only 24%. However, in a second experiment in 1992, root and stem

14   growth reductions in the 2×-ambient treatment were similar (65% versus 60%) (Neufeld et al.,

15   1995). In Finland, reduced root growth was found for a number of clones of silver birch

16   (Oksanen and Saleem, 1999). After 5 years, root growth was decreased by 33%, but shoot

17   growth was not affected by O_3 exposures of a 7-h mean of 15 ppm-h over 5 years in a FACE

18   system (Oksanen, 2001). When first-year poplar seedlings (*P. tremuloides*) were exposed in

19   OTCs to two O_3 concentrations and six N concentrations, the root/shoot ratio was decreased soon

20   after exposure to O_3 in all N treatments, even though O_3 effects on total biomass were not

21   detected in the low-N and very high-N treatments (Pell et al., 1995). These results suggest that

22   effects on the root/shoot ratio occur before significant growth effects arise. In a series of OTC

23   experiments lasting 1 to 3 years at 3 different elevations in Switzerland, fine root growth in

24   European beech was found to be more sensitive to O_3 than was shoot or total biomass (Braun and

25   Fluckiger, 1995). Although the estimated effect of O_3 on fine root biomass was similar to that

26   for total biomass, fine root biomass was significantly decreased at AOT40 (24-h) values of only

27   3 ppm·h, while total biomass was not significantly decreased until AOT40 values reached 30 to

28   40 ppm·h.

29

9.6.5.3   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). As discussed for annual plants above (Section 9.6.4.6), Level I critical values are not designed for making quantitative estimates of the O_3 effects on vegetation at the regional scale. For long-lived perennials, additional problems complicate extrapolation. As discussed below (Section 9.6.5.7), 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 exposure (Broadmeadow, 1998).

9.6.5.4   Summary of Effects on Deciduous Woody Species

Recent evidence from free air exposure systems and OTCs supports results observed previously in OTC studies (Table 9-16, Figure 9-1). 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 aspen clones using OTCs (Karnosky et al.,

1   1999). New evidence is also available comparing various aspects of O_3 sensitivity between

2   seedlings and mature trees of some species, notably red oak. As has been observed in previous

3   criteria documents, root growth is often found to be the most sensitive indicator in terms of

4   biomass response to O_3.

5   Results since 1996 support the conclusions of the 1996 AQCD (U.S. Environmental

6   Protection Agency, 1996) that individual deciduous trees are generally less sensitive to O_3 than

7   are most annual plants, with the exception of a few genera such as *Populus*, which are highly

8   sensitive. However, the data presented in Table 9-19 suggest that ambient exposures that occur

9   in different regions of the United States can sometimes reduce the growth of seedlings of

10   deciduous species. Results from multiple-year studies sometimes show a pattern of increasing

11   effects in subsequent years. Although, in some cases, growth decreases due to O_3 become less

12   significant or even disappear over time. While some mature trees show greater O_3 sensitivity in

13   physiological parameters such as net photosynthetic rate compared to seedlings, these effects

14   may not translate into measurable reductions in biomass growth. Because even multiple-year

15   experiments do not expose trees to O_3 for more than a small fraction of their life span and

16   because competition may, in some cases, exacerbate the effects of O_3 on individual species,

17   determining the effects on mature trees remains a significant challenge. Effects on mature trees

18   under natural conditions are discussed after the review of evergreen species below and more

19   fully in Section 9.7, in the context of extrapolating from controlled studies to forest ecosystems.

20

21   **9.6.5.5 Evergreen Woody Species**

22   Most investigations have shown evergreen tree species to be less sensitive to O_3 compared

23   to deciduous species (U.S. Environmental Protection Agency, 1996). For example, exposure-

24   response functions based on 23 experimental cases of seedling response to O_3, suggest that a

25   SUM06 exposure for 3 months of 42.6 ppm·h would protect evergreen species from a 10%

26   growth loss in 50% of the cases (Table 9-16). For deciduous species, the corresponding SUM06

27   value was 31.5 ppm·h (Table 9-16). As another example, experiments in the Great Smoky

28   Mountains National Park found black cherry seedlings to demonstrate substantial decreases in

29   biomass, as discussed above and shown in Table 9-19 (Neufeld et al., 1995). However, exposure

30   for up to three growing seasons did not decrease the biomass of eastern hemlock, Table

1   Mountain pine, and Virginia pine seedlings exposed to O_3 under similar conditions in this

2   location, as shown in Table 9-19 (Neufeld et al., 2000).

3   As for deciduous species, there is a substantial range in sensitivity among evergreen

4   species. As discussed above for deciduous species, a risk analysis was undertaken to predict tree

5   biomass growth reductions due to O_3 based on exposure-response equations for tree seedlings

6   combined with species distribution across the eastern United States and interpolated O_3 exposure

7   (Hogsett et al., 1997). While some species such as Virginia pine were predicted to be affected

8   only slightly even in a high O_3 year, the growth of sensitive evergreen species such white pine

9   was predicted to be reduced by 5% in a lower-than-average O_3 year and 10% in a high ozone

10   year across 50% of its range (Andersen et al., 1997). The remainder of this section discusses

11   experimental results for evergreen species in the order shown in Table 9-20.

12   Douglas fir seedlings were exposed to elevated O_3 concentrations in a free air zonal air

13   pollution system in British Columbia, Canada for two growing seasons with 12-h mean values in

14   1988 of 18 to 41 ppb and in 1989 of 27 to 66 ppb (Runeckles and Wright, 1996). Although

15   substantial variation was seen in effects among the different treatments, there was a significant

16   decrease in the growth of the second flush weight in the second year, with reductions of 55% at

17   the highest O_3 exposure, based on a linear regression. This result contrasts with the lack of

18   effect seen in a previous study with seedlings of this species grown in pots for 134 days and

19   exposed to 7-h mean O_3 concentrations up to 71 ppb (Table 9-30 in U.S. Environmental

20   Protection Agency, 1996).

21   First-year loblolly pine seedlings of 53 open-pollinated families were exposed to

22   1×-ambient O_3 in OTCs for a single growing season, and average growth volume was decreased

23   by 14% compared to a CF treatment (McLaughlin et al., 1994). The 1×-ambient O_3 exposure in

24   this study expressed as 24-h SUM00 was 137 ppm·h, and the CF treatment reduced O_3 by 47%.

25   In this study, the root/shoot ratio was decreased significantly in six of the nine families

26   examined. Exposure to O_3 with SUM06 values up to 162 ppm·h and 132 ppm·h in 2 successive

27   years in OTCs had no effect on seedlings grown in competition with various species of grasses

28   and forbs (Barbo et al., 2002). Exposure of 3-year-old seedlings to O_3 exposures of up to

29   2.5×-ambient (12-h mean of 98 ppb) also had no significant effect (Anttonen et al., 1996).

30   Information summarized in the 1996 AQCD (U.S. Environmental Protection Agency, 1996),

31   indicated that significant effects on seedling growth were observed in several studies of

1   seedlings exposed to elevated O_3 concentrations for one or more years. Several studies,

2   including that of McLaughlin et al. (1994), demonstrate considerable variation in O_3 sensitivity

3   among different genotypes of loblolly pine.

4   For ponderosa pine seedlings, the 1996 AQCD reviewed a number of studies with

5   exposures to elevated O_3 concentrations for one to three growing seasons (U.S. Environmental

6   Protection Agency, 1996). Recent similar studies support the earlier results (Table 9-20);

7   (Andersen et al., 2001; Takemoto et al., 1997). The 1996 criteria document also discussed at

8   some length the ongoing work examining effects of O_3 across a naturally-occurring O_3 gradient

9   in the San Bernardino Mountains in California. Since that time, much research on ponderosa

10 pine has focused on interactive effects of additional stresses such as nitrogen, and effects of O_3

11 on physiological parameters (Sections 9.4, 9.7). Effort has also focused on the effects of O_3 on

12 root growth because such effects could alter sensitivity to drought or nutrient stress. Ecosystem

13 level effects of ozone are discussed further in Section 9.7, but some information relevant to

14 exposure-response relationships is discussed below.

15   For several tree species, O_3 has been shown in experimental studies with seedlings to

16 reduce root growth more than shoot growth (U.S. Environmental Protection Agency, 1996).

17 Ponderosa pine has been shown to be sensitive to O_3, and studies with seedlings have shown

18 reduced root growth, decreases root/shoot ratios, and decreased allocation to roots (Andersen

19 et al., 1991; 1997; Andersen and Rygiewicz, 1995; Andersen and Scagel, 1997; Tingey et al.,

20 1976). More recently, data from a long-studied pollution gradient in the San Bernardino

21 Mountains of southern California suggests that O_3 substantially reduces root growth in natural

22 stands of ponderosa pine. Root growth in mature trees was decreased at least 87% in a high

23 pollution site as compared to a low pollution site (Grulke et al., 1998), and a similar pattern was

24 found in a separate study with whole tree harvest along this gradient (Grulke and Balduman,

25 1999). Because other potential influences on root growth, including shading by competing trees,

26 soil temperature, soil moisture, phenology, were not correlated with the observed pattern of

27 reduced root growth, the authors conclude that O_3 was the cause of the observed decline in root

28 growth. Further results of field investigations with ponderosa pine and other pine species native

29 to California are discussed below under the heading "Scaling experimental data on long-lived

30 species to field conditions" as well as in Section 9.7.

1   Table Mountain pine, Virginia pine, and eastern hemlock seedlings were exposed to

2   various levels of O_3 (CF to 2 × ambient) in OTCs for in a series of experiments two or three

3   years in Great Smoky Mountains National Park in Tennessee (Neufeld et al., 2000). There were

4   no statistically significant effects of O_3 exposure on stem or root biomass, and only slight effects

5   on the biomass of the oldest needles in Table Mountain pine in the 2× ambient treatment.

6   As reviewed in the 1996 criteria document, studies of the response of red spruce to

7   O_3 exposures generally have not found effects on growth of seedlings or saplings, even after

8   exposure to high concentrations (12-h mean of 90 ppb) for up to 4 years. A more recent report

9   confirms that this slow-growing species is O_3 insensitive for at least several years (Laurence

10   et al., 1997).

11   For perennial vegetation, cumulative effects over more than one growing season may be

12   important. For three-year-old Norway spruce in Sweden, exposure to elevated O_3 for three

13   growing seasons decreased total biomass by 18% and stem biomass by 28% (Karlsson et al.,

14   1995). However, after a fourth season of exposure, total biomass decreased significantly by only

15   8% (Karlsson et al., 2002). In this experiment, the O_3 exposures expressed as 12-h mean values

16   averaged over four growing seasons were 12 and 44 ppb for the CF and 1.5×-ambient treatments,

17   respectively; and AOT40 values were 2 and 23 ppm·h, respectively. Despite 4 years of

18   exposure, this experiment did not demonstrate a consistent trend in the O_3 effect on biomass to

19   indicate a significant carry-over effect.

20

21   **9.6.5.6   Summary of Effects on Evergreen Woody Species**

22   In summary, the O_3 sensitivity of different genotypes within species and between species

23   of evergreen vegetation varies widely. Based on studies with seedlings in OTCs, major species

24   in the United States are generally less sensitive than are most deciduous trees, and slower-

25   growing species are less sensitive than faster-growing ones. However, exposure to ambient O_3

26   may reduce the growth of seedlings of commonly occurring species. Because tree species are

27   long-lived, most experiments have only covered a very small portion of the life span of a tree,

28   making estimating of any effect on mature trees difficult. Considerations for scaling the results

29   of seedling studies to mature forest trees as well as additional information from field surveys and

30   studies of mature trees under natural conditions are discussed below and in Section 9.7.

31

1   **9.6.5.7 Scaling Experimental Data to Mature Trees**

2   As compared with annual crop species, it is much more difficult to define appropriate

3   exposure-response relationships for tree species. For annual species, an experiment may cover

4   the whole life span of the plant, but it is not feasible to provide controlled-exposure conditions

5   for long-lived plants for any significant portion of their life spans. Most studies have used small

6   seedlings because they are manageable under experimental conditions; but seedlings and mature

7   trees may have different sensitivities to O_3. For perennial species, effects of O_3 may accumulate

8   over more than 1 year, and may interact with other stresses such as drought stress over multiple

9   growing seasons. As for annual species (Section 9.4.2), substantial variability occurs among

10   evergreen genotypes and this variation may interact with other stress responses differently in

11   different landscapes and regions. Despite these difficulties, investigators have addressed some

12   of these issues since the 1996 AQCD (U.S. Environmental Protection Agency, 1996). New

13   information is available on the response of mature evergreen trees to O_3 under field conditions,

14   and models based on tree physiology and stand dynamics have been used to predict O_3 effects on

15   forest stands and regions. The following issues are reviewed briefly below: (1) interaction of

16   drought and O_3 stress, (2) scaling data from seedlings to mature-tree studies. Two additional

17   scaling issues are addressed in Section 9.7: (1) scaling data to forest stands, and (2) scaling data

18   to ecosystems and regions.

19

20   *9.6.5.7.1 Interactive Effects of Drought and Ozone*

21   Many interacting factors may influence the effect of O_3 on vegetation. For crop plants,

22   environmental conditions are often managed such that nutrients and water are not strongly

23   limiting; but for native vegetation, including most perennial species, such factors are likely to

24   limit growth. The effects of interacting stresses on vegetation are reviewed in Section 9.4.

25   However, because drought is common in many forests, and because there is substantial evidence

26   that it alters the response of trees to O_3, it is discussed in this section in the context of

27   determining exposure-response relationships for trees.

28   Controlled experiments with seedlings provide direct evidence that drought can reduce the

29   impact of O_3. For example, for 3-year-old Norway spruce in Sweden, exposure to elevated

30   O_3 for three growing seasons decreased total biomass by 18% and stem biomass by 28%

31   (Karlsson et al., 1995). However, for droughted trees, both total and stem biomass decreased

1   only 5%, with a statistically significant interaction with O_3 for stem biomass. Yet after a fourth

2   season of exposure, there was no longer any interaction between drought and O_3, while there was

3   a significant decrease of 8% in the biomass when both drought and well-watered data were

4   combined (Karlsson et al., 2002). In this study, seedlings were grown in sand in 120 L pots and

5   for the drought treatment, water was withheld for 4 weeks during the first year and for 7 to

6   8 weeks during each of the last 3 years. In this experiment, the O_3 exposures expressed as 12 h

7   seasonal daylight mean averaged over four growing seasons were 12 and 44 ppb for the CF and

8   1.5×-ambient treatments respectively. Over this period, the AOT40 values for the treatments

9   averaged 2 and 23 ppm·h respectively. Despite 4 years of exposures, this experiment did not

10   demonstrate a consistent trend in drought O_3 interactions. The difference in effects seen between

11   the third and fourth season suggest that scaling drought-O_3 interactions from seedlings to mature

12   trees may be difficult. However, evidence from biomonitoring surveys supports an interaction

13   between drought and O_3 effects, at least for foliar symptoms. In systematic surveys of foliar

14   symptoms on species selected as biomonitors throughout much of the eastern United States,

15   symptoms were more common and more severe in areas with high O_3 concentrations (Smith

16   et al., 2003). However, in very dry years, such as 1999, the occurrence and severity of

17   symptoms was greatly reduced, even in areas with high ambient O_3 concentrations.

18

19   ### 9.6.5.7.2 *Scaling from Seedlings to Mature Trees*

20   Because most experiments are conducted with seedlings, various methods are required to

21   scale experimental data on seedlings to mature trees. An overview of physiological differences

22   between young and old plants, and the consequences of these differences for O_3 sensitivity, is

23   provided in Section 9.4.5.3. The discussion below focuses on information relevant to developing

24   exposure-response relationships for mature trees. Information from a few experimental studies,

25   as well as scaling efforts based on physiological characteristics incorporated into models, are

26   discussed in Section 9.7.

27   Although most studies continue to examine the effects of O_3 on seedlings, during the 1990s

28   some studies examined the effects of O_3 on the response of mature trees. Studies of mature trees

29   demonstrate differences in some aspects of O_3 sensitivity between seedlings and mature trees.

30   For some species, such as red oak, seedlings are less sensitive to O_3 than are mature trees

31   (Hanson et al., 1994; Samuelson and Edwards, 1993; Wullschleger et al., 1996). Both red oak

1   seedlings and genetically related mature trees were exposed to CF, 1×-ambient, or 2×-ambient

2   O_3 exposures in OTCs in Tennessee for two growing seasons (Hanson et al., 1994). Nine large

3   chambers (4.6 × 8.2 m) were used to enclose individual mature trees and standard EPA-style

4   OTCs were used for potted seedlings. Ozone exposures expressed as a 24-h SUM00 were 34,

5   79, and 147 ppm·h in 1992 and 37, 95, 188 ppm·h in 1993 for the sub-ambient, and 2×-ambient

6   treatments. Mature trees had a greater light-saturated net photosynthetic rate and stomatal

7   conductance compared to seedling foliage at physiological maturity. By the end of the growing

8   season, exposure to 1×-ambient and 1×-ambient O_3 reduced the light-saturated net

9   photosynthetic rate and stomatal conductance of mature trees by 25 and 50%, respectively,

10 compared with the CF treatment (35 ppm·h). In seedlings, however, light-saturated net

11 photosynthetic rate and stomatal conductance were less affected by O_3 exposure. The authors

12 concluded that extrapolations of the results of seedling-exposure studies to foliar responses of

13 mature forests without considering differences in foliar anatomy and stomatal response between

14 juvenile and mature foliage may introduce large errors into projections of the O_3 responses of

15 mature trees.

16   In a study of ponderosa pine in California, seedlings and branches of mature trees

17 (in branch chambers) were exposed to O_3 concentrations of 0.5-, 1-, and 2×-ambient O_3

18 concentrations (Momen et al., 1997). Net photosynthetic rate of 1-year-old, but not current-year,

19 foliage was reduced in mature trees while not significantly reduced in seedlings. This effect was

20 not due to alteration of stomatal conductance by O_3. This result contrasts with those with earlier

21 studies of red spruce (Rebbeck et al., 1993).

22   In contrast to the findings for red oak and ponderosa pine, giant sequoia seedlings had

23 higher rates of stomatal conductance, CO_2-exchange rate, and dark respiration than did mature

24 trees (Grulke and Miller, 1994). As compared to older trees, stomatal conductance was more

25 than 7-fold greater in current-year and 4-fold greater in 2-year-old seedlings (Grulke and Miller,

26 1994). The authors concluded that giant sequoia seedlings are sensitive to atmospheric O_3 until

27 ~5 years of age. Low conductance, high water use efficiency, and compact mesophyll all

28 contribute to a natural O_3 tolerance, or to O_3 defense, or to both, in the foliage of older trees.

29 Similarly, lower stomatal conductance was found in mature Norway spruce in Austria compared

30 to seedlings grown with optimal water and nutrients in a growth chamber (Wieser, 1997). In this

31 study, net photosynthetic rate was less sensitive to added O_3 in mature trees compared to

1   seedlings. In a related study, the average rate of O_3 uptake of 17-year-old trees

2   ~0.6 nmol m^{-2} s^{-1}, decreasing linearly in older trees, such that rates were only ~0.1 m^{-2} s^{-1} in

3   216-year-old trees (Wieser et al., 1999).

4   Based on a review of studies of stomatal conductance in both seedlings and mature trees,

5   Samuelson and Kelly (2001) concluded that O_3 uptake in oak species, black cherry, sugar maple,

6   and American beech averaged 47% lower in potted seedlings than in mature trees. For evergreen

7   species, they concluded that O_3 uptake in seedlings averaged 26% higher than in mature trees.

8   They also suggested that artifacts introduced by growth in pots confound these differences that

9   exposure-response functions derived from seedlings grown in situ are more applicable to mature

10   trees than are studies of seedlings grown in pots (Samuelson and Kelly, 2001).

11   As discussed above for annual vegetation, it has long been noted that internal O_3 dose is

12   more appropriate than external O_3 exposure for assessing the effects of O_3 on vegetation, because

13   effects occur primarily via the uptake of O_3 through the stomata (Section 9.3.2). However,

14   external O_3 exposure sometimes has been shown to explain O_3 effects as well or better than

15   calculated internal O_3 dose. For ponderosa pine, Grulke and others (2002) found little difference

16   in the response of net photosynthetic rate and stomatal conductance to O_3 exposure as compared

17   to calculated O_3 uptake; and estimated O_3 uptake by ponderosa pine and O_3 exposure at several

18   sites were highly correlated ($r^2 = 0.92$). For red oak, Hanson and others (1994) found that

19   SUM00 explained 83% of the variance in the response of light-saturated photosynthetic rate to

20   O_3 levels, while estimated internal dose explained only 76% of the variance. In this same study,

21   SUM06 explained only 49% of the variance. Due to genetic variation or other factors, individual

22   mature trees will vary in their response to similar O_3 exposures. For example, in 125-year-old

23   giant sequoia trees exposed to ~230 ppm·h of O_3 in branch chambers, O_3 uptake in one

24   individual was ~5 mmol m^{-2}, while in another it was ~9.5 mmol m^{-2} (Grulke et al., 1996).

25   Based on these results, stomatal conductance, O_3 uptake, and O_3 effects cannot be assumed

26   to be equivalent in seedlings and mature trees. In general, mature deciduous trees are likely to

27   be more sensitive to O_3 compared to seedlings, while mature evergreen trees are likely to be less

28   sensitive than seedlings. However, even when differences in physiological traits occur,

29   concomitant effects on stem growth may not be detected in the field. Additionally, complex

30   interactions may occur between environmental conditions and O_3 responses;and artifacts may

31   occur for seedling studies, especially for seedlings grown in pots. Section 9.7 further discusses

1   issues that must be addressed when scaling data from individual mature trees to forests and

2   regions.

3

4   **9.6.6   Studies with the Chemical EDU**

5   The chemical EDU (*N*-[2-(2-oxo-1-imidagolidinyl)ethyl]-*N*-phenylurea) has been used

6   with the goal of protecting plants from O_3 effects without controlling O_3 exposure (Table 9-21)

7   (U.S. Environmental Protection Agency, 1986, 1996). The use of EDU has the potential to be a

8   low-cost, practical method of evaluating ambient O_3 exposures on plants grown under natural

9   conditions without the limitations imposed by methodologies such as OTCs (Section 9.2). EDU

10   can be applied easily as a foliar spray or a soil drench and, with more effort, can be injected

11   directly into plant stems. However, because EDU is phytotoxic, and may have effects on plants

12   other than antioxidant protection, it is crucial that the correct dosage for protection from O_3, be

13   determined without the direct effects of EDU. Unfortunately, although many studies with EDU

14   have been conducted in recent decades, very few have used multiple EDU application levels

15   along with multiple O_3 exposures to characterize the EDU system for a given plant species.

16   Therefore, the text of this section focuses on how data from existing studies can be used for

17   developing or validating exposure-response relationships, rather than reviewing results of all

18   individual studies. Data from individual studies on O_3 exposure, EDU application rates, and the

19   effects of EDU are presented in Table 9-21. In addition to EDU, sodium erythorbate has been

20   used in a few studies as a protectant chemical. Since very few published studies have used

21   sodium erythorbate and attempts to establish appropriate doses for individual species are even

22   more limited, the use of this chemical is not reviewed here.

23   The phytotoxicity of EDU is well known, and the point has been made repeatedly that for

24   a particular species or cultivar, tests under a range of environmental conditions and O_3 exposures

25   must be made to establish the efficacy of EDU for quantifying O_3 effects (Heggestad, 1988;

26   Kostka-Rick and Manning, 1992). A recent study has shown that even low concentrations of

27   EDU (8 to 32 mg L^{-1} soil), decreased bean yield under low O_3 exposure (7-h mean of 19 ppb) in

28   CF OTCs (Miller et al., 1994). This study also demonstrated that phytotoxicity (both foliar

29   symptoms and growth effects) can differ even in the same series of experiments, apparently due

30   to changes in environmental conditions, and that EDU can suppress yield at application rates that

31   do not always cause foliar symptoms (Miller et al., 1994). Finally, this study found that EDU

1   altered biomass partitioning by increasing vegetative growth and decreasing reproductive

2   growth. A study of bean grown in OTCs in Germany found that EDU treatment in CF OTCs

3   significantly increased yield, while EDU had no significant effect on yield in other O_3 treatments

4   (Brunschon-Harti et al., 1995). In this study, O_3 significantly reduced the mass of pods, shoots,

5   and roots. EDU increased root, leaf, and shoot mass across O_3 treatments. However, there was

6   only a significant interaction with O_3 for root mass. This study indicates that EDU can stimulate

7   above-ground growth and/or delay senescence regardless of O_3 treatment. Together, these

8   studies suggest that EDU has effects other than its antioxidant protection and phytotoxicity.

9   Recent studies have also shown that EDU does not always have greater effects at higher O_3

10   exposures. For bean grown in pots in studies in Spain and the Netherlands, EDU increased pod

11   yield (Ribas and Penuelas, 2000; Tonneijck and Van Dijk, 1997). However, this effect was not

12   greater at sites with higher O_3 exposure despite consistent experimental protocols at all sites,

13   including growing the same cultivar in pots with adequate water (Ribas and Penuelas, 2000;

14   Tonneijck and Van Dijk, 1997). Such results suggest that it may be difficult to quantify

15   ambient O_3 effects using EDU, because the amount of plant growth or yield expected at a low

16   (background) O_3 concentration cannot be inferred from EDU-treated plants grown at locations

17   with higher O_3 exposures.

18   Potted white clover exposed to ambient O_3 at 12 sites throughout Europe over

19   three growing seasons was evaluated in a meta-analysis (Ball et al., 1998). A soil drench of

20   100 mL EDU was applied every 2 weeks for 3 months per growing season. Only very weak

21   evidence of a linear relationship of the ratio of biomass in control versus EDU treated plants

22   across all sites ($r^2 = 0.16$) was seen. However, an artificial neural network (ANN) model

23   including VPD, temperature, longitude, year, and altitude explained much more of the variance

24   ($r^2 = 0.79$). The authors suggested that the greater sensitivity at certain sites in Germany may

25   have been due to occurrence of other pollutants. This meta-analysis indicates that EDU effects

26   may be influenced substantially by environmental factors.

27   In summary, the EDU method for assessing the impact of ambient O_3 exposures is

28   potentially useful, because it provides a separate line of evidence than other methods. However,

29   as has been pointed out by numerous authors, the system must be carefully characterized for

30   each species under different environmental conditions and different O_3 exposures.

31   Unfortunately, such characterization has so far been limited, although substantial progress has

1   been made for radish (Kostka-Rick et al., 1993; Kostka-Rick and Manning, 1992, 1993). Effects

2   of EDU can include phytotoxicity and alteration of biomass partitioning, along with differential

3   effects on the same species in different years or locations. Additionally, the degree of O_3

4   protection afforded by a certain application of EDU is difficult to quantify. Thus, it is difficult to

5   use data from existing EDU studies to develop exposure-response relationships or to quantify the

6   effects of ambient O_3 exposure. Despite these limitations, the EDU studies reviewed in previous

7   criteria documents (U.S. Environmental Protection Agency, 1986, 1996) and the more recent

8   studies summarized in Table 9-21 provide another line of evidence that ambient O_3 exposures

9   occurring in many regions of the United States may be reducing the growth of crops and trees.

10

11   9.6.7   Summary

12   Recently published data support the conclusions of previous criteria documents that there

13   is strong evidence that ambient concentrations of O_3 cause injury and damage to numerous

14   common and economically valuable plant species. For annual vegetation, the data summarized

15   in Table 9-17 show a range of growth and yield responses both within and among species.

16   Nearly all of these data were derived from studies in OTCs, with only two studies using open-air

17   systems in the UK (Ollerenshaw et al., 1999; Ollerenshaw and Lyons, 1999). It is difficult to

18   compare studies that report O_3 exposure using different indices, such as AOT40, SUM06, or 7-h

19   or 12-h mean values. However, when such comparisons can be made, the results of recent

20   research confirm earlier results summarized in the 1996 AQCD (U.S. Environmental Protection

21   Agency, 1996). A summary of earlier literature concluded that a 7-h, 3-month mean of 49 ppb

22   corresponding to a SUM06 exposure of 26 ppm·h would cause 10% loss in 50% of 49

23   experimental cases (Tingey et al., 1991). Recent data summarized in Table 9-17 support this

24   conclusion, and more generally indicate that ambient O_3 exposures can reduce the growth and

25   yield of annual species. Some annual species such as soybean are more sensitive, and greater

26   losses may be expected (Table 9-17). Thus the recent scientific literature supports the

27   conclusions of the 1996 AQCD (U.S. Environmental Protection Agency, 1996) that ambient O_3

28   concentrations are probably reducing the yield of major crops in the United States.

29   Much research in Europe has used the AOT40 exposure statistic, and substantial effort has

30   gone into developing Level-1 critical levels for vegetation using this index. Based on regression

31   analysis of 15 OTC studies of spring wheat including one study from the United States and

1   14 from locations ranging from southern Sweden to Switzerland, an AOT40 value of 5.7 ppm·h

2   was found to correspond to a 10% yield loss, and a value of 2.8 ppm·h corresponded to a 5%

3   yield loss (Fuhrer et al., 1997). Because a 4 to 5% decrease could be detected with a confidence

4   level of 99%, a critical level of an AOT40 value of 3 ppm·h was selected in 1996 (Kärenlampi

5   and Skärby, 1996).

6   In addition to likely reductions in crop yield, O_3 may also reduce the quality or nutritive

7   value of annual species. Many studies have shown effects of O_3 on various measures of plant

8   organs that affect quality, with most studies focusing on characteristics important for food or

9   fodder. These studies indicate that there may be economically important effects of ambient O_3

10 on the quality of crop and forage species. Previous criteria documents have concluded that

11 visible symptoms on marketable portions of crops and ornamental plants can occur with seasonal

12 7-h mean O_3 exposures of 40 to 100 ppb (U.S. Environmental Protection Agency, 1978, 1986,

13 1996). The recent scientific literature does not refute this conclusion.

14   The use of OTCs may reverse the usual vertical gradient in O_3 that occurs within a few

15 meters above the ground surface (Section 9.2). This reversal suggests that OTC studies may

16 somewhat overestimate the effects of an O_3 concentration measured several meters above the

17 ground. However such considerations do not invalidate the conclusion of the 1996 AQCD (U.S.

18 Environmental Protection Agency, 1996) that ambient O_3 exposures (Tables 9-14 and

19 Table 9-22) are sufficient to reduce the yield of major crops in the United States.

20   As for single-season agricultural crops, yields of multiple-year forage crops are reduced at

21 O_3 exposures that occur over large areas of the United States. This result is similar to that

22 reported in the 1996 AQCD (U.S. Environmental Protection Agency, 1996). When species are

23 grown in mixtures, O_3 exposure can increase the growth of O3- tolerant species and exacerbate

24 the growth decrease of O_3-sensitive species (e.g., Ashmore and Ainsworth, 1995; Fuhrer et al.,

25 1994). Because of this competitive interaction, the total growth of the mixed-species community

26 may not be affected by O_3 exposure (Ashmore and Ainsworth, 1995; Barbo et al., 1998; Fuhrer

27 et al., 1994). However, in some cases, mixtures of grasses and clover species have shown

28 significant decreases in total biomass growth in response to O_3 exposure in studies in the United

29 States (Heagle et al., 1989; Kohut et al., 1988) and in Sweden (Pleijel et al., 1996). In Europe,

30 a provisional critical level for herbaceous perennials of an AOT40 value of 7 ppm·h over

31 6 months has been proposed to protect sensitive plant species from adverse effects of O_3.

1   For deciduous tree species, recent evidence from free air exposure systems and OTCs

2   supports results observed previously in OTC studies. For example, a series of studies

3   undertaken using free air O_3 enrichment in Rhinelander, WI (Isebrands et al., 2000, 2001)

4   demonstated that O_3-symptom expression was generally similar in OTCs, FACE, and also sites

5   along an ambient O_3 gradient, supporting the previously observed variation among aspen clones

6   using OTCs (Karnosky et al., 1999). As has been observed in previous criteria documents, root

7   growth often is found to be the most sensitive biomass response indicator to O_3.

8   Results reported since 1996 support the conclusion of the 1996 AQCD (U.S.

9   Environmental Protection Agency, 1996) that deciduous trees are generally less sensitive to

10   O_3 than are most annual plants, with the exception of a few very sensitive genera such as

11   *Populus* and sensitive species such as black cherry. However, the data presented in Table 9-19

12   suggest that ambient O_3 exposures that occur in the United States can potentially reduce the

13   growth of seedlings of deciduous species. Results from multiple-year studies sometimes show a

14   pattern of increased effects in subsequent years. In some cases, however, growth decreases due

15   to O_3 may become less significant or even disappear over time. While some mature trees show

16   greater O_3 sensitivity in physiological parameters such as net photosynthetic rate than do

17   seedlings, these effects may not translate into measurable reductions in biomass growth.

18   However, because even multiple-year experiments do not expose trees to O_3 for more than a

19   small fraction of their life span, and because competition may in some cases exacerbate the

20   effects of O_3 on individual species, determining effects on mature trees remains a significant

21   challenge.

22   In Europe, a Level I critical level has been set for forest trees based on OTC studies of

23   European beech seedlings. A critical level was defined as an AOT40 value of 10 ppm·h for

24   daylight hours for a 6-month growing season (Kärenlampi and Skärby, 1996). However, other

25   studies show that some species such as silver birch may be more sensitive to O_3 compared to

26   beech (Pääkkönen et al., 1996).

27   For evergreen tree species, as for other tree species, the O_3 sensitivity of different

28   genotypes and different species varies widely. Based on studies with seedlings in OTCs, major

29   species in the United States are generally less sensitive than are most deciduous trees, and

30   slower- growing species are less sensitive than are faster-growing species. Interacting stresses

31   such as competition stress may increase the sensitivity of trees to O_3. As for deciduous species,

1 most experiments with evergreen species have only covered a small portion of the life span of a

2 tree and have been conducted with seedlings, making estimating effects on mature trees difficult.

3   For all types of perennial vegetation, cumulative effects over more than one growing

4 season may be important, furthermore, studies for only a single season may underestimate

5 effects. Mature trees may be more or less sensitive to O_3 than are seedlings, depending on the

6 species, but information on physiological traits may be used to predict some such differences.

7 In some cases, mature trees may be more sensitive to O_3 than seedlings due to differences in

8 their gas exchange rates, growth rates, greater cumulative exposures, or due to the interaction of

9 O_3 stress with other stresses.

10

11

12 ## 9.7 EFFECTS OF OZONE EXPOSURE ON NATURAL ECOSYSTEMS

13 ### 9.7.1 Introduction

14   The preceding section on species-level responses (9.6) provides a lead-in to address the

15 response of ecosystems to ozone (O_3). The conclusion of the 1996 O_3 AQCD was that aside

16 from the results from the San Bernardino Forest, there was no direct evidence that O_3 is altering

17 natural ecosystems in the United States. This conclusion is generally valid today, except that our

18 understanding of the effects of O_3 in the San Bernardino forest has been tempered by additional

19 understanding of the complicating role that N deposition plays in this system. Despite the lack

20 of any new, direct information linking O_3 with ecosystem changes, numerous publications since

21 1996 have highlighted ways in which O_3 may affect ecosystem structure and/or function. This

22 section addresses new and (where appropriate) older literature in order to illustrate possible

23 shifts in energy or material flow through ecosystems as a result of O_3 exposure.

24   An ecosystem is defined as comprising all of the organisms in a given area interacting with

25 the physical environment, so that a flow of energy leads to a clearly defined trophic structure,

26 biotic diversity, and cycling of materials between living and nonliving parts (Odum, 1963).

27 Individuals within a species and populations of species are the building blocks from which

28 communities and ecosystems are constructed. Classes of natural ecosystems are distinguished

29 by their dominant vegetation form, e.g., tundra, wetland, deciduous forest and conifer forest.

30 Boundaries of ecosystems are delineated when an integral unit is formed by the physical and

1   biological parts. Defined pathways for material transport and cycling, and for the flow of energy
2   are contained within the integrated unit.

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

9   Ecosystems are functionally highly integrated. Thus, changes in one part of an ecosystem,
10   such as the primary producer component, may have consequences for connected parts, such as
11   the consumer and decomposer components. For example, when needles are shed prematurely as
12   a result of O_3 exposure, successional development of phyllosphere fungi inhabiting the surface of
13   ponderosa pine needles may be truncated (Bruhn, 1980). In addition, decomposer populations in
14   the litter layer may be capable of higher rates of decomposition, because the younger age classes
15   of needles falling from O_3-damaged pines have higher nitrogen (N) content (Fenn and Dunn,
16   1989). Because ecological systems integrate the effects of many influences, the resulting effect
17   of O_3 exposure may depend on co-occuring influences that predispose an ecosystem to stress
18   (Colls and Unsworth, 1992). One important change in our thinking since the 1996 O_3 AQCD is
19   that at high levels of O_3 exposure that are known to result in detectable plant responses
20   (> 250 ppm h accumulated over a growing season), N deposition must also be considered as a
21   concurrent stressor. Changes in N cycling and compartmentalization in the ecosystem result
22   from both O_3 exposure and increased N deposition.

23   The vast majority of O_3 effects literature addresses individual species responses (see
24   Section 9.7.4.3), which was also true in 1996 (U.S. Environmental Protection Agency, 1996).
25   This section differs from the preceding one in that physiological stress of individual species is
26   considered only within the context of the natural ecosystem. Changes in function at the level of
27   the individual are propagated through higher levels of organization, resulting in changes in
28   ecosystem structure and function. However, since ecosystem-level responses result from the
29   interaction of organisms with one another and with their physical environment, it takes longer
30   for a change to develop to a level of prominence that can be identified and measured. The
31   paucity of scientific literature on O_3 effects at the ecosystem level is a result of both the

1   complexity of ecological systems, and long response times. In addition, "indirect" effects of O_3

2   on plants (e.g., affecting the plants' ability to integrate environmental stresses) may be more

3   important than direct effects on photosynthesis and respiration at the leaf level (Johnson and

4   Taylor, 1988).

5   A conceptual framework (see Table 9-22) suitable for organizing discussion of the effect of

6   O_3 on ecosystems was developed by the EPA Science Advisory Board (Young and Sanzone,

7   2002). Their six essential ecological attributes (EEAs) include landscape condition, biotic

8   condition, chemical and physical characteristics, ecological processes, hydrological and

9   geomorphological processes, and natural disturbance regimes (listed as subsection headings

10   below and in Table 9-22). The major ecological effects, and gaps in our knowledge of O_3

11   exposure effects at the ecosystem level are summarized at the end of this chapter. While the

12   main focus is O_3 effects newly described since the last Ozone Criteria Document (EPA, 1996),

13   many key historical papers are cited to demonstrate ecosystem response, particularly where they

14   remain the only examples in the literature. Although the vast majority of published studies focus

15   on individuals, five field examples and one FACE experiment have measured several ecosystem

16   components simultaneously to better understand ecosystem response to O_3. We provide an

17   overview of these six studies up-front because they provide a context for the subsequent

18   discussion on possible ecosystem effects.

19

20   **9.7.2 Case Studies**

21   **9.7.2.1 Valley of Mexico**

22   The first evidence of air pollution impacts on vegetation in the Valley of Mexico (Mexico

23   City Air Basin) were observations of foliar injury symptoms in bioindicator plants attributed to

24   O_3, PAN, SO_2 and possibly other pollutants (de Bauer, 1972). Subsequently, O_3 injury to foliage

25   and crowns of pine trees were reported in forests to the south and southwest of Mexico City

26   (Krupa and de Bauer, 1976; de Bauer and Hernández-Tejeda, 1986). Ozone is considered to be

27   the pollutant with the most severe impacts on vegetation within the Mexico City urban zone and

28   in forests downwind of the city. *P. hartwegii* is the most O_3-sensitive pine species and is

29   severely impacted by high O_3 exposures encountered to the south/southwest of the metropolitan

30   area (Miller et al., 2002). The potential for O_3 injury is particularly high in this area, because O_3

31   levels are high during the summer rainy season when soil moisture availability and stomatal

Table 9-22. Essential Ecological Attributes for Natural Ecosystems Affected by O₃

Category	Species	Condition Measures	References
Landscape Condition • Habitat Types			
Biotic Condition • Ecosystems and Communities	Mixed conifer forest	Community composition, Stand structure	Miller et al. (1989); Miller and McBride (1999)
Community Extent Community Composition	*Pinus ponderosa*	Relative abundance	Miller (1973); Arbaugh et al. (2003)
	Grassland communities	Species composition	Ashmore and Ainsworth (1995); Ashmore et al. (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 & 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)
Diseases	*Populus hybrids*	Septoria occurrence	Woodbury et al. (1994)
	Populus hybrids	Rust occurrence	Beare et al. (1999)
	Populus tremuloides	Rust occurrence	
	Populus tremuloides	Forest tent caterpillar/ paratisoid performance	Karnosky et al. (2002); Percy et al. (2002) Holton et al. (2003)
	Picea abies & Picea sitchensis	Needle fungi	Magan et al. (1995)
	Pinus ponderosa	Root disease x O₃ interactions	Fenn et al. (1990)
	Pinus taeda	Canker dimensions	Carey and Kelly (1994)
	Pinus sylvestris/ mycorrhizae	Disease susceptibility	Bonello et al. (1993)
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	O₃ sensitivity	Andersen et al. (2001)
	*Pinus taeda/*diverse community	Tree growth	Barbo et al. (2002)

Table 9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

Category	Species	Condition Measures	References
• Species and Populations			
Population Size	*Pinus strobus* *Pinus ponderosa*	Mortality Mortality	Karnosky (1981) Carroll et al. (2003)
Genetic Diversity/ Population Structure	*Lupinus bicolor* *Populus tremuloides*	% population sensitive % population sensitive	Dunn (1959) Berrang et al. (1986, 1989, 1991)
	Trifolium repens *Plantago major*	% population sensitive % population sensitive	Heagle et al. (1991) Reiling and Davison (1992a); Davison and Reiling (1995); Lyons et al. (1997)
Population Dynamics	*Trifolium repens* *Plantago major*	Adaptation Population changes over time	Heagle et al. (1991) 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)
	Pinus jeffreyi	Foliar symptoms	Patterson and Rundel (1995); Salardino and Carroll (1998); Grulke et al. (2003b)
	Prunus serotina	Foliar symptoms	Fredericksen et al. (1995, 1996); Chappelka et al. (1997, 1999a,b); Hillebrand et al. (1996); Ghosh et al. (1998); Lee et al. (1999); Ferdinand et al. (2000, 2003);
	Liriodendron tulipfera	Foliar symptoms	Yuska et al. (2003) Somers et al. (1998); Hillebrand et al. (1996);
	Sassafras albidum	Foliar symptoms	Chappelka et al. (1999a)
	Populus nigra, *Fraxinus excelsior &* *Prunus avium*	Foliar symptoms	Chappelka et al. (1999a) Novak et al. (2003)
	Fagus sylvatica	Foliar symptoms	Gerosa et al. (2003); Vollenweider et al. (2003a)
	Fraxinus americana	Foliar symptoms	Schaub et al. (2003); Ferdinand 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 & *Verbesina occidentalis*	Foliar symptoms	Chappelka et al. (2003)
	Asclepias incarnata	Foliar symptoms	Orendovici et al. (2003)

Table 9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

Category	Species	Condition Measures	References
• Physiological Status	*Pinus halepensis*	Allometry	Welburn 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. (1998); Grulke and Balduman, (1999)
	Festiva ovina	Root/shoot ratio	Warwick and Taylor, (1995)
	Betula pubescens	Root/shoot ratio	Mortensen (1998)
	Fagus sylvatica	Root/shoot ratio	
	Populus tremuloides x *P. tremula*	Root/shoot ratio	Paludan-Müller et al. (1999); Landolt et al. (2000)
	Prunus serotina	Leaf area	Oksanen et al. (2001a)
	Fraxinus excelsior	Leaf area	
	Populus tremuloides	Leaf area index	
	Pinus ponderosa	Carbon allocation to mycorrhizae	Neufeld et al. (1995) Wiltshire et al. (1996)
	Betula pendula	Decreased winter bud formation	Karnosky et al. (2003a) Andersen and Rygiewicz (1995)
	Betula pendula	Delayed bud break	Oksanen (2003a,b)
	Acer saccharum	Early bud break	
			Prozherina et al. (2003) Bertrand et al. (1999)
• Reproductive Status	*Apocynun androsaemifolium*	Flowering time 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 x ananassa	Fruit yield	
	Plantago major	Seed yield	Drogoudi and Ashmore (2000, 2001) Reiling and Davison (1992b); Pearson et al. (1996); Whitfield et al. (1997); Lyons and Barnes (1998)
	Understorey herbs	Seed yield	Harward and Treshow (1975)

Table 9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O$_3$

Category	Species	Condition Measures	References
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. (1995b); 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 & radial growth	Kelting et al. (1995)
	Quercus rubra	Root turnover	Coleman et al. (1996)
	Populus tremuloides	Soil respiration	King et al. (2001)
	Populus tremuloides	Soil respiration	Andersen and Scagel (1997);
	Pinus ponderosa	Soil respiration	Scagel and Andersen (1997), Andersen (2000) Samuelson and Kelly (1996) Coleman et al. (1995b)
	Quercus rubra	Carbon partitioning & allocation	Andersen et al. (1997); Grulke et al. (1998);
	Populus tremuloides	Carbon allocation	Grulke and Balduman (1999);
	Pinus ponderosa	Carbon allocation	Grulke et al. (2001)
	Betula pendula	Carbon allocation	Karlsson et al. (2003)
	Betula pendula	Carbon allocation	Oksanen and Saleem, (1999); Salem et al. (2001)
	Fragaria vesca	Carbon allocation	Manninen et al. (2003)
	Pinus taeda	Root respiration	Edwards (1991)
	Lespedeza cuneata & Schizacbyrium scoparium	Yield	Powell et al. (2003) Somers et al. (1998)
	Liriodendron tulipfera	Radial growth	Vollenweider et al. (2003b)
	Prunus serotina	Radial growth	Peterson et al. (1987)
	Pinus jeffreyi	Radial growth	Peterson et al. (1993)
	Pinus ponderosa	Radial growth (no effect)	Bartholomay et al. (1997)
	Pinus strobus	Radial growth	and Downing (1995, 1996)
	Pinus taeda	Radial growth	Braun et al. (1999)
	Fagus sylvatica	Stem volume	Wallin et al. (2002)
	Picea abies	Stem volume	Isebrands et al. (2001)
	Populus tremuloides	Volume growth	Andersen et al. (1991)
	Pinus ponderosa	Root growth	

Table 9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O_3

Category	Species	Condition Measures	References
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	Reiling and Davison (1992a); Davison and Reiling (1995); Lyons et al. (1997); 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 (1999)
	Fagus sylvatica	Relative growth rate	Bortier et al. (2000)
	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 & Betula papyrifera*	Altered foliar C:N ratio and N resorption efficiency	Lindroth et al. (2001)
	Andropogon virginicus & Rubus cuneifolius	Litter decomposition rate	Kim et al. (1998)
	Liriodendron tulipera	Litter decomposition rate	Scherzer and Rebbeck (1998) Findlay and Jones (1990)
	Populus deltoides	Litter decomposition rate	Fenn and Dunn (1989)
	Pinus ponderosa	Litter decomposition rate	Kainulainen et al. (2003)
	Pinus sylvestris	Litter decomposition (no effect)	
Nitrogen Cycling	*Pinus ponderosa*	Altered foliar N	Momen and Helms (1996)
		Foliar N & O_3 exposure (no effects)	Bytnerowicz et al. (1990)
	Pinus taeda		Mandersheid et al. (1992)
	Prunus serotina & Liriodendron tulipfera	Altered foliar N metabolism Altered foliar N	Boerner and Rebbeck (1995)
Other Nutrient Cycling	*Picea sitchensis & Pinus sylvestris*	Foliar leaching (no effect)	Skeffington and Sutherland (1995)
	Pinus ponderosa	Nutrient availability & O_3	Bytnerowicz et al. (1990)

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• Water Budget	*Picea rubens*	Water-use efficiency (no effect)	Laurence et al. (1997)
	Pinus armandi	Water-use efficiency	Shan et al. (1996)
	Pinus jeffreyi	Canopy transpiration	Grulke et al. (2003a)
	Picea abies	Transpiration, xylem sap flow	Maier-Maercker (1997)
	Fraxinus excelsior	Stem flow of water	Wiltshire et al. (1994)
	Betula pendula	Water-use efficiency	Maurer and Matysek (1997)
	Populus hybrids	Water-use efficiency	Reich and Lassoie (1984)

Table 9-22 (cont'd). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

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 (1999)
• Extent	*Pinus ponderosa*	Extent of bark beetle attack	Minnich et al. (1995)
• Duration	*Pinus ponderosa*	Duration of bark beetle attack	Minnich et al. (1995)

Source: Young and Sanzone (2002.

1 conductance are greatest and these factors enhance O₃ uptake and injury. Decline of *A. religiosa*

2 (oyamel) in the Desierto de los Leones National Park is a well-known example of dramatic

3 dieback and mortality of entire forest stands due primarily to air pollution stress

4 (Alvarado-Rosales and Hernández-Tejeda, 2001). Other factors, such as a lack of stand

5 thinning, also contribute to forest decline. Lead in automobile gasoline was phased out in 1990,

6 and foliar concentrations of heavy metals in forest species are not now at phytotoxic levels (Fenn

7 et al. 2001a). Sulfur dioxide concentrations decreased in the early 1990s as a result of regulatory

8 mandates limiting their emissions. Sensitive plants in the NE and NW sectors of the Mexico

9 City urban zone where concentrations are highest may still be impacted by exposure to ambient

10 SO₂ levels. Deposition of ionic forms of N and S are high in forested areas southwest of Mexico

11 City. The effects of these chronic nutrient inputs to the forest are only beginning to be

12 investigated and understood.

1   The ecological perturbations caused by severe air pollution exposures in forests located

2   downwind of Mexico City are expected to continue for the near future (the next 5 to 10 years),

3   largely as a result of high O_3 concentrations, as well as of N oxides emissions. The longer-term

4   response is more uncertain, and depends largely on the effectiveness of regulatory emissions

5   control strategies. Currently, pollutant levels are declining. Forest responses to this trend will

6   depend on how long it takes to reduce levels sufficiently to allow sensitive species to recover.

7   Some of the change to the ecosystem is probably irreversible, such as the loss of lichen diversity

8   and of other O_3-sensitive species (Zambrano et al., 2002).

9

10   **9.7.2.2   San Bernardino Mountains**

11   The San Bernardino Mountains lie east of the Los Angeles Air Basin (California, USA),

12   and significant levels of pollution have been transported into the mountain range, including a

13   Class I Wilderness area. The effects of O_3 exposure on the mixed conifer forest of the

14   San Bernardino Mountains is perhaps the longest and most thoroughly documented O_3

15   ecological effects evaluation (Miller and McBride, 1999). In this classic case study linking

16   tropospheric O_3 exposure to damage to an entire forest ecosystem (U.S. Environmental

17   Protection Agency, 1996) (Table 9-23), Miller et al. (1963) first identified the unique foliar

18   chlorotic mottle that was occurring on two of the dominant tree species, *Pinus ponderosa* and

19   *P. jeffreyi*. Levels of O_3 averaging 100-120 ppb over 24 hours with 1-hour peaks well into the

20   200 ppb range were common in the region in the 1960s and 1970s (Miller and McBride, 1999).

21   Single hour peak values have declined in recent years due to heavily regulated pollution control,

22   but O_3 concentrations in the moderate range continue to rise and accumulate, increasing the

23   overall cumulative exposure (Arbaugh et al., 1998; Takemoto et al., 2001; Lee et al., 2003).

24   Since the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) was written, the

25   concurrent role of N deposition in modifying ecosystem response to O_3 exposure in the San

26   Bernardino Mountains has been further elucidated (Fenn et al., 1996, 2003; Bytnerowicz et al.,

27   1999; Takemoto et al., 2001; Bytnerowicz, 2002). Both O_3 exposure and N deposition reduce

28   foliar retention (Grulke and Balduman, 1999) and alter tissue chemistry of both needles and litter

29   (Poth and Fenn, 1998). In addition, confounding factors such as drought and fire suppression

30   add to the complexity of ecosystem response (Minnich et al., 1995; Takemoto et al., 2001;

31   Arbaugh et al., 2003). Extensive crown injury measurements have also been made, linking

Table 9-23. 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; Krupa and Bauer, 1976; Hernández-Tejeda, 1986) • Community composition changes (Alvarado-Rosales and Hernández-Tejeda, 1986) • Species richness changes (Zambrano et al., 2002)
San Bernardino Mountains	*Pinus ponderosa, P. jeffreyi*	Field transects	40 yrs	• Community composition changes (Miller, 1973; Minnich et al., 1995; Arbaugh et al., 2003) • Population changes (McBride and Laven, 1999) • O$_3$/pine/bark beetle interaction (Pronos et al. 1999) • Altered C flows (Grulke et al., 2002b; Grulke et al., 1998; Grulke and Balduman, 1999; Grulke et al., 2001; Arbaugh et al., 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 (Miller and Millecan, 1971; Miller et al,. 1972) • Link to decreased growth (Peterson et al., 1987, 1991, 1995) • Quantification of O$_3$ flux (Bauer et al., 2000; Panek et al., 2002; Goldstein et al., 2003) • 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 9-23 (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 (Percy et al., 2002; Holton et al., 2003) • C & N cycling (Lindroth et al., 2001; King et al., 2001) • Moderation of CO$_2$ responses by O$_3$ (Isebrands et al., 2001; Wustman et al., 2001; McDonald et al., 2002; Karnosky et al., 2003a)
Plantago	*Plantago major*	Field	20 yrs	• Population structure (Davison and Reiling, 1995; Lyons et al., 1997) • O$_3$ resistance (Reiling and Davison, 1992a) • Adaptation (Davison and Reiling, 1995)
Carpathian Mountains	*Pinus sylvestris, Picea abies*	Field	15 yrs	• Significant foliar injury • Community composition changes • Species diversity changes

1   ambient O$_3$ exposure data to chlorotic mottle and fascicle retention (Arbaugh et al., 1998).

2   Ozone exposure and N deposition reduce carbon allocation to stems and roots (Grulke et al.,

3   1998; 2001), further predisposing trees to drought stress, windthrow, root diseases, and insect

4   infestation (Takemoto et al., 2001). Increased mortality of susceptible tree species (*ponderosa*

5   and *Jeffrey pine*, *Pinus ponderosa*, *P. Jeffreyii*) has shifted community composition towards

6   white fir and incense cedar (Abies concolor, Libocedrus decurrens) and has altered forest stand

7   structure (Miller et al., 1989). Ozone exposure is implicated in projected changes in stand

8   composition (McBride and Laven, 1999) toward a predominance of oaks, rather than mixed

9   conifer forests. Forest understory species have also been affected (Temple, 1999). These

10   individual species responses collectively have affected trophic structure and food web dynamics

11   (Dahlsten et al., 1997; Pronos et al., 1999), as well as C and N cycling (Arbaugh et al., 2003)

12   (Table 9-24). Because of the high N deposition in the San Bernardino Mountains, it is difficult

13   to separate out effects of only O$_3$ versus those due to the combined effects of O$_3$ and N

14   deposition (Table 9-25).

Table 9-24. The Most Comprehensively Studied Effects of O_3 on Natural Ecosystem is the San Bernardino Mountain Forest Ecosystem. Citations Focus on Research Published Since U.S. EPA (1996).

Pollutant Occurrence	Reference
O_3 exposures N deposition	Grulke et al. (1998); Fenn et al. (1996, 2000, 2003); Bytnerowicz et al. (1999)
Cellular, Biochemical	
Foliar pigments Antioxidants	Grulke and Lee (1997); Grulke (1999); Tausz et al. (1999a,b 2001); Tausz et al. (1999a,b,c, 2001)
Foliar Responses	
Foliar Symptoms	Grulke and Lee (1997); Arbaugh et al. (1998); Miller and Rechel (1999)
Gas Exchange	
Photosynthesis & Conductance O_3 flux Foliar nutrients	Grulke (1999); Grulke and Retzlaff (2001); Grulke et al. (2002a,b)
Whole Organism	
Growth/Biomass • Aboveground • Belowground • Root/shoot ratio • Carbon allocation • Crown vigor	Grulke and Balduman (1999) Grulke et al. (1998); Grulke and Balduman (1999) Grulke and Balduman (1999) Grulke et al. (2001) Arbaugh et al. (1998); Miller and Rechel (1999)
Ecosystem	
Community dynamics/succession Simulations	McBride and Miller (1999); Arbaugh et al. (2002) Arbaugh et al. (1999)
Understory vegetation	Temple (1999)
Pest interactions • Bark beetle/predators • Disease occurrence • Litter decomposition	Dahlsten et al. (1997); Pronos et al. (1999) Miller and Rechel (1999); Pronos et al. (1999)
Disturbance	
• Bark beetle occurrence • Fire frequency	Minnich et al. (1995); Minnich (1999); Miller and McBride (1999)

Table 9-25. 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	Tausz et al. (2001); Grulke et al. (2003b)
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)
á Carotenoids	i	n.d.	d	Grulke et al. (2003b)
Foliar nitrogen	d	i	d	Grulke and Lee (1997); Grulke et al. (1998); 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	Takemoto et al. (1997); Grulke and Lee (1997; Grulke et al. (1998, 2003b); Grulke (1999); Tausz et al. (2001)
Gas Exchange				
A_{max} lower canopy	n.d.	i	d	Grulke (1999); Grulke and Retzlaff (2001); Grulke et al. (2002b); Panek (2004)
A_{max} whole canopy	d	n.d.	d	Panek and Goldstein (2001); Grulke et al. (2003b); Misson et al. (2004)
A_{max} seedlings	di	n.d.	n.d.	Grulke and Retzlaff (2001)
Stomatal limitation	á to ps	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 et al. (2002a; Grulke (1999)
O_3 flux	d	n.s.	d	Panek and Goldstein (2001); Panek et al. (2002; 2003); Grulke et al. (2002b; 2004)

Table 9-25 (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. (1998)
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 ozone, 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.

1   **9.7.2.3   Sierra Nevada Mountains**

2   The western slope of the Sierra Nevada Mountains in central and southern California has

3   also been exposed to elevated O_3 for a long time, although the effects have been much less than

4   those observed in the San Bernardino Mountains. Symptoms of O_3 injury have been found on

5   ponderosa and Jeffrey pines in all of the Sierra Nevada National forests and parks (Carroll et al.,

6   2003). First identified as a problem in the 1970s (Miller et al., 1972), elevated O_3 with daytime

7   means of 60-80 ppb are common (Böhm et al., 1995; Bauer et al., 2000; Bytnerowicz et al.,

8   2002a; Panek et al., 2002). The west-slope Sierra Nevada forests are also exposed to a wide

9   range of additional gaseous and particulate pollutants, including various S and N compounds

1   (Bytnerowicz et al., 1999; Takemoto et al., 2001; Fenn et al., 2003), but at levels much lower

2   than in the San Bernardino Mountains. Typical O_3-induced visible foliar symptoms, including

3   chlorotic mottle, chlorophyll degradation, and premature senescence, are commonly found on

4   sensitive genotypes of *ponderosa pine* (Peterson et al., 1991; Arbaugh et al., 1998; Staszak et al.,

5   2003) and *Jeffery pine* (Peterson et al., 1987; Patterson and Rundel, 1995; Arbaugh et al., 1998;

6   Grulke et al., 2003b). Other important conifers in the region, such as giant sequoia, appear to be

7   relatively tolerant to O_3 (Grulke et al., 1996). The symptoms of foliar injury and growth

8   reductions have been verified on seedlings in O_3 exposure chambers (Temple, 1988; Momen and

9   Helms, 1996; Momen et al., 2002).

10   Ozone foliar injury of dominant pine species in the Sierra Nevada Mountains is correlated

11   to decreased radial growth in both *ponderosa pine* (Peterson et al., 1991) and *Jeffreyi pine*

12   (Peterson et al., 1987; Patterson and Rundel, 1995). Because of the large amount of intraspecific

13   variation in O_3 sensitivity in these two species, O_3 exposure may be a selective agent (Patterson

14   and Rundel, 1995), with differential mortality rates for sensitive individuals (Carroll et al.,

15   2003). The region's forests may also be experiencing subtle changes in species composition and

16   community structure (Patterson and Rundel, 1995; Takemoto et al., 2001).

17   Based on fire scar dating, reconstructions of stand age classes, historical records, and

18   present stand structure, fire has been largely excluded in western forests for the last 75 to 100

19   years (Minnich, 1999; Minnich and Padgett, 2003). Fire exclusion has resulted in fewer, large,

20   stand-replacing fires rather than a mosaic of smaller, lower intensity fires. The change in fire

21   intensity may have selectively altered stand structure, fitness and competitiveness of component

22   species, and their susceptibility to atmospheric pollutants and other stressors (Minnich, 1999).

23   Short-lived (50-80 years) species such as knobcone and Coulter pine, which occur at the

24   interface between the chaparral and the mixed conifer forest, may already have been selected for

25   O_3 tolerance due to seedling establishment (the most sensitive tree age class in conifers) after

26   large fires in the 1950's (Minnich, 1999). Strong measures to suppress fires have largely kept

27   chaparral fires from invading the mixed conifer forests, and stand densification in the mixed

28   conifer zone has increased. High stand density, in turn, may weaken the younger cohorts and

29   increase sensitivity to atmospheric pollution (Minnich, 1999).

30   Other disturbances that play a potential role in sensitivity to atmospheric pollution include

31   cycles of drought stress. Nearly every decade is marked by one or more years of very low

1   precipitation (Graumlich, 1993). During extended periods of drought, foliar injury is lower than

2   in subsequent years with higher average precipitation (Carroll et al., 2003). In the first several

3   years (1975-1977) of a Sierran-wide assessment of O_3 injury to pines, O_3 injury increased,

4   because of greater water availability due to greater stomatal conductance and, presumably,

5   greater O_3 uptake. Trees instrumented with monitors to directly measure canopy transpiration

6   had 20% greater stomatal conductance in mesic microsites (riparian areas, mid-slope seeps) than

7   trees in xeric microsites (rock outcrops) (Grulke et al., 2003a). Although the Sierra Nevada

8   experienced a prolong drought between 1987 and 1993, it was less severe and O_3 injury did not

9   significantly decrease (Carroll et al., 2003). The same plots showed only a slight increase in O_3

10 injury between 1993 and 2000. Drought stress, in general, can make trees more susceptible to

11 insect and pathogen infestation. However, serious outbreaks of insect infestation are believed to

12 be indicators, not a cause, of existing stress in the forest (Wickman, 1992).

13

14 **9.7.2.4 Appalachian Mountains**

15   The southern Appalachian Mountain region experiences some of the highest O_3 exposures

16 of any natural areas in the eastern United States (Mueller, 1994; Hildebrand et al., 1996;

17 Samuelson and Kelly, 1997; Chappelka et al., 1997). Since the region is the home of the

18 Shenandoah and Great Smokey Mountains National Parks, which have Class I air quality

19 designations by the 1977 Clean Air Act, there has been considerable study of the region's

20 dominant forest species to determine O_3 effects. Visible foliar symptoms of O_3 have been found

21 in natural ecosystems consisting of *Sassafras albidum* (Chappelka et al., 1999a), *Prunus serotina*

22 (Hildebrand et al., 1996; Chappelka et al., 1997; 1999b; Samuelson and Kelly, 1997),

23 *Liriodendron tulipifera* and *Fraxinus Americana* (Hildebrand et al., 1996; Chappelka et al.,

24 1999a). Visible foliar symptoms induced by O_3 have been recreated on the same species in

25 chamber studies (Duchelle et al., 1982; Chappelka et al., 1985; Chappelka and Chevone, 1986;

26 Samuelson, 1994; Fredericksen et al., 1995). No response to O_3 exposure has been found for

27 other hardwood trees, nor for the three conifer species tested (Neufeld et al., 2000).

28   Long term foliar injury symptoms have been correlated with decreased radial growth in

29 tulip poplar and cherry (*Liriodendron tulipifera* and *Prunus serotina*; Somers et al. (1998) and

30 with decreased biomass in cherry (Neufeld et al., 1995). Although climatic conditions (drought)

31 largely explained radial growth reductions, O_3 exposure may have also contributed (McLaughlin

1   and Downing, 1996). Ozone exposure may also be affecting the understory vegetation in the

2   region (Duchelle and Skelly, 1981; Duchelle et al., 1983; Chappelka et al., 1997; Chappelka

3   et al., 2003; Davison et al., 2003) and community composition (Barbo et al., 1998), through

4   impacts both on growth and reproduction (Chappelka, 2002). Foliar litter from trees exposed to

5   elevated O_3 have lower decomposition rates (Kim et al. 1998). Other air pollutants are likely to

6   be found in this ecosystem but not at such high deposition values found in the California studies.

7   A decline in forest health in the northern Appalachians has been primarily attributed to the

8   effects of acidic fog and rain on soil acidification, lower Ca^{2+} availability, reduction in fine root

9   biomass, and modification of cuticular wax. However, fog- and O_3-exposed red spruce forests

10   also show winter injury (Percy, 2002).

11

12   **9.7.2.5 Plantago Studies in the United Kingdom**

13   One of the most well-documented studies of population and community response to O_3

14   effects are the long-term studies of *Plantago major* in native plant communities in the United

15   Kingdom (Reiling and Davison, 1992a; Davison and Reiling, 1995; Lyons et al., 1997).

16   Sensitive populations of *P. major* had significant growth decreases in elevated O_3 (Reiling and

17   Davison, 1992b and 1992c; Pearson et al., 1996; Whitfield et al., 1997) and reduced fitness as

18   determined by decreased reproductive success (Reiling and Davison, 1992b; Pearson et al.,

19   1996). While spatial comparisons of population responses to O_3 are complicated by other

20   environmental factors, rapid changes in O_3 resistance were imposed by ambient levels and

21   variations in O_3 exposure (Davison and Reiling, 1995). Molecular patterns of genetic variation

22   suggest that change in O_3 resistance over time probably resulted from selection on genotypes

23   already present in local populations, rather than through an influx of new *P. major* germplasm

24   (Wolff et al., 2000). The highest correlations between O_3 resistance and ambient O_3

25   concentrations occurred at the site of seed collection (Lyons et al., 1997), rather than between O_3

26   resistance and other climatic variables, as was found for aspen (Berrang et al., 1991).

27

28   **9.7.2.6 Forest Health in the Carpathian Mountains**

29   The Carpathian Mountains cross five countries (the Czech Republic, the Slovak Republic,

30   Poland, Romania, and the Ukraine) and contain many national parks and several biosphere

31   reserves. The forests were largely cleared in the 15th century, and were then planted in Norway

1   spruce plantations. As elevation increases, beech (*Fagus sylvatica*) or beech-fir (*Abies alba*)

2   forests grade into Norway spruce (*Picea abies*) or spruce-fir forests. Near the treeline, Norway

3   spruce combines with dwarf mountain pine (*Pinus mugo*). Dwarf mountain pine forms an

4   almost pure stand just below alpine vegetation.

5   The forests of the Carpathian Mountains have been subjected to anthropogenic stressors

6   (shepherding, metal mining, wood harvest for structures and paper) for hundreds of years, as

7   described for the Tatra Mountains in the southern Carpathians (Wezyk and Guzik, 2004). The

8   Carpathians have been subjected to regional air pollution stresses since industrialization. Most

9   of the effects of air pollution on forest health degradation were due to (1) heavy metal

10   deposition, (2) soil acidification by acid deposition, and (3) subsequent pest outbreaks, the

11   combination of which led to the forest decline and dieback between 1970 and 1989 (Dunajski,

12   2004). By the 1980's, industrial pollutants such as SO_2 and heavy metals significantly declined,

13   but O_3 exposure has continued to increase (Bytnerowicz et al., 2004). Increased ownership and

14   use of private cars in Central Europe, as well as long-range transport of O_3 from western Europe,

15   are believed to be responsible for the continued increase in photooxidants. In 1995, drought

16   resulted in significant mortality, as well as an epidemic of bark beetle infestation in subsequent

17   years. A network of air quality monitoring sites was installed across Europe in the late 1980's, as

18   part of the International Cooperative Programme on Assessment and Monitoring of Air Pollutant

19   Effects on Forests (ICP Forests). Mean defoliation rates for six important forest species across

20   Europe have increased or remained unchanged from 1989 to 1999 (Percy, 2002). Ozone

21   concentrations experienced in the Tatra Mountains, especially along the southern slopes,

22   occasionally reach 190-200 ppb as two-week-long averages, with the highest values experienced

23   in early summer at elevations of 1700 to 2300 m (Bytnerowicz et al., 2004). In other parts of the

24   Carpathian Mountains, peak two-week averages of O_3 concentrations were lower, at 160 ppb

25   (Bytnerowicz et al., 2002). For all trees inventoried, about 13% exhibited greater than 25%

26   defoliation during 1997 to 2000. There was no difference between extent of damage for

27   broadleaves or conifers. Trees in Poland and the Czech Republic are the most affected by air

28   pollution, and the least damaged forests are in Romania (Badea et al., 2002).

29   The extent to which O_3 exposure affects forest health degradation, and slows forest

30   degradation, is still unknown in Europe. In many of the published studies, response to a known

31   O_3 gradient is largely confounded by other pollutants and/or climatic gradients (Bytnerowicz

1   et al., 1996; Szaro et al., 2002; Widadki et al., 2004). Current levels of ambient O_3 are believed

2   to be high enough to reduce bole radial growth (Percy, 2004). Although average O_3

3   concentration alone was not related to bole growth, the peak hourly O_3 concentration was

4   negatively correlated to growth (Muzika et al., 2002). Recent evidence indicates that canopy

5   health of European white oak (*Quercus robur*), Norway spruce, maritime pine (*Pinus pinaster*),

6   and beech has significantly declined (Huttunen et al., 2002). However, the canopy health of

7   Scots pine (*Pinus sylvestris*) has improved. The network of air quality monitoring stations and

8   forest plots is extensive and active. Subsequent correlative analyses including both

9   meteorological and air quality attributes throughout the EU will help to determine the specific

10 role of O_3 exposure in forest decline. Historical effects of anthropogenic disturbance may still

11 be confounding.

12

13 **9.7.2.7 Field Exposure System (FACE), Rhinelander, Wisconsin**

14   The Aspen Free-Air CO_2 Enrichment (FACE) facility was designed to examine the effects

15 of both elevated CO_2 and O_3 on aspen (*Populus tremuloides*), birch (*Betula papyfera*), and sugar

16 maple (*Acer saccharum*) in a simple reconstructed plantation characteristic of Great Lakes

17 Aspen-dominated forests (Karnosky et al., 1999, 2003a). Instead of using chambers to expose

18 the plants to desired gas concentrations, the gas is piped up vertical delivery tubes in the open

19 air. The vertical delivery pipes surround a 30-m diameter circular plot with five different aspen

20 clones in half of the plot, one quarter of the plot planted in aspen and birch, and one quarter in

21 aspen and maple. The O_3 treatment for the first five years was 1.5× ambient, with ambient O_3

22 exposures averaging 35 to 37 ppb (12 h daytime over the growing season) compared to elevated

23 O_3 rings averaging 49 to 55 ppb for the same time period (Karnosky et al., 2003a).

24   Elevated CO_2, elevated O_3, and elevated $CO_2 + O_3$ have had effects on most system

25 components being measured in the study (Table 9-26; Karnosky et al., 2003a). One interesting

26 finding of the project has been the nearly complete offset by elevated O_3 of the enhancements

27 induced by elevated atmospheric CO_2 for the pioneer keystone species *P. tremuloides* (Isebrands

28 et al., 2001) and *B. papyrifera* (Percy et al., 2002), even though O_3 exposure alone did not

29 always result in a significant response when compared to controls. They also found evidence

30 that the effects on above- and below-ground growth and physiological processes have cascaded

31 through the ecosystem, even affecting microbial communities (Larson et al., 2002; Phillips et al.,

Table 9-26. 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 Three Years of Treatments at the Aspen FACE Project (Modified from Karnosky et al. 2003a)

	CO$_2$	O$_3$	CO$_2$ + O$_3$	Reference
Foliar Gene Expression and Biochemistry				
Rubisco; RbcS[2] transcripts	d[1]	d	dd	Wustman et al. (2001); Noormets et al. (2001a)
PAL transcipts	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.	Lindroth et al. (2001); Kopper and Lindroth (2003a,b)
Tannins	n.s.	i	i	Lindroth et al. (2001); Kopper and Lindroth (2003a,b)
Foliar nitrogen	d	n.s.	d	Lindroth et al. (2001); Kopper and Lindroth (2003a,b)
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	Takeuchi et al. (2001); Noormets et al. (2001a)
A$_{max}$ whole canopy	ii	dd	n.s.	Noormets et al. (2001b); Sharma et al. (2003)
Stomatal limitation	d	n.s.	d	Noormets et al. (2001a)
Stomatal conductance	d	di	d	Noormets et al. (2001a)
Foliar respiration	n.s.	i	n.s.	Takeuchi et al. (2001); Noormets et al. (2001b)
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. (2001b); Takeuchi et al. (2001); Wustman et al. (2001)
O$_3$ flux	d	ii	i	Noormets et al. (2001a)
Growth and Productivity				
Leaf thickness	i	n.s.	n.s.	Oksanen et al. (2001b)
Leaf size	i	d	d	Wustman et al. (2001)
Leaf area	i	d	n.s.	Noormets et al. (2001b)

1   2002). This study also confirmed earlier observations of changes in trophic interactions

2   involving keystone tree species, as well as important insect pests and their natural enemies

3   (Table 9-26) (Percy et al., 2002; Holton et al., 2003; Awmack et al., 2003).

4

9.7.3 Landscape Condition

6   In the SAB framework (Figure 9-19), landscape condition is assessed using the areal

7   extent, composition of component landscape ecosystems or habitat types, and the pattern or

8   structure of component ecosystems or habitat types (including biocorridors). To date, no

9   publications exist on the impacts of O_3 exposure on landscape condition. The effects of O_3

10   exposure have only been reported at the community or stand level (see Biotic Conditions,

11   below). The following is a description of current discussions by land stewards and of how

12   difficult it will be to quantitatively assess the effect of O_3 exposure on landscape condition.

13   Landscapes are identified and preserved, such as National Parks, Class I Wilderness Areas,

14   etc., so that they are protected from the effects of O_3 exposure by law. Efforts to determine

15   whether landscapes have been affected by certain levels of exposure rely on valuation of

16   landscape and ecosystem components. Several different approaches of valuation have been

17   used, such as pathological (visible symptoms), biomass and allocation, and biogeochemical.

18   In the pathological approach, a "critical loads" concept is developed, with varying levels of

19   impact viewed as acceptable, interim targets, or as unacceptable. As an example, land managers

20   of Class I Wilderness Areas may consider a critical level acceptable if it resulted in no visible O_3

21   symptoms to sensitive species. In concrete terms, sensitive species may respond to peak O_3

22   exposures of 60 ppb (e.g., coneflower, Rudbeckia laciniata, in Great Smoky National Park;

23   Davison et al., 2003), and so the critical exposure level would be < 60 ppb for any hourly valued

24   during the growing season. An interim target would be that less than 5% of the sensitive plants

25   would have visible symptoms of < 15% of the leaf surface. An unacceptable level of O_3

26   exposure would be any result more pronounced than the interim target. The advantage of the

27   foliar injury approach is that large crews with relatively simple training can assess individual

28   species within the landscape and "see" the effect of the oxidant exposure. There are several

29   disadvantages, however. Some species (e.g., white fir) exhibit no foliar injury, but do have shifts

30   in biomass allocation in response to oxidant exposure (Retzlaff et al., 2001). Other species have

31   shown significant decreases in foliar injury due to needle loss, retranslocation of nutrients to

Sources
Above-ground Processes
(−) Rubisco activity
(+) Lipid Peroxidation
(−) Stomatal Control
(−) Phloem Loading
(+) Senescence

Sinks
Above-ground Processes
(+) Antioxidant Synthesis
(+) Repair Processes
(+) Construction Costs
(+) Tissue Respiration
(−) Growth
(±) Reproduction

Storage Carbohydrates
(−) Pool Size
(−) Concentration

Storage Carbohydrates
(−) Pool Size
(±) Concentration

Root Storage
(−) Pool Size
(−) Concentration

Root Processes
(−) Growth
(±) Mycorrhizal
      Colonization
(?) Turnover

Root Storage
(−) Pool Size
(−) Concentration

Figure 9-19. A conceptual diagram of processes and storage pools in sources and sinks that are affected by ozone 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 ozone action occurs in the leaves (from Andersen, 2003).

1   remaining foliage, with subsequent increased photosynthetic rate (Beyers et al., 1992).

2   In addition, the development of foliar symptoms within a species is related to sunlight and

3   microclimate (Davison et al., 2003).

4   In the biomass approach, O$_3$ exposure resulting in a measureable decline in biomass

5   (usually of a target, sensitive species) is used to evaluate landscape condition. The bulk of the

6   information available is from seedling responses to controlled chamber exposures, reviewed in

7   the previous section. Some information exists for species in natural environments, but teasing

8   out concurrent stressors and finding adequate "controls" may be intractable. For example, in a

1   long-term gradient of O_3 exposure, N deposition, and drought, the site with the highest O_3

2   exposure had the greatest whole tree biomass (pole-sized trees) due to growth stimulation by N

3   deposition (Grulke and Balduman, 1999).

4   In the biogeochemical approach, changes in biogeochemical cycling are used to assess

5   landscape condition. Species sensitive to O_3 exposure have well known responses to O_3

6   exposure, including altered C allocation to below- and above-ground tissues, and altered rates of

7   leaf production, turnover, and decomposition. Changes in turnover rates of ephemeral tissues

8   (leaves, fine roots) also affect nutritional status of the remaining tissue. These shifts can affect

9   overall carbon and N loss from the ecosystem in terms of respired C, and leached aqueous

10   dissolved organic and inorganic C and N. Instability in C and N pools and dynamics can affect

11   landscape-level nutrient dynamics even without significant inputs of N deposition. The endpoint

12   assessment is based on changes in water quality from or in the landscape, correlated to a defined

13   oxidant exposure level. These approaches are linkable: visible injury at a particular level could

14   be related to reduction in photosynthate, which would reduce whole plant biomass (and carbon

15   dynamics). If O_3 sensitive species are dominant within the landscape, then changes in C and N

16   dynamics over time would be expected to alter biogeochemical cycles. Examples of forest types

17   that contain geographically extensive, O_3-sensitive species that could be used in assessing

18   landscape-level changes include ponderosa pine in the western United States, tulip poplar

19   (*Liriodendron tulipifera*) or loblolly pine (*Pinus taeda L.*) in the eastern U.S. decidous forests,

20   and Norway spruce (*Picea abies (L.) Karst*) in the Carpathian Mountains of eastern Europe.

21   Water quantity may also be affected by O_3 exposure at the landscape level. Moderately

22   high O_3 exposure may affect the mechanism of stomatal opening (McAinsh et al., 2002),

23   resulting in sluggish stomatal opening and closing (Patterson and Rundel, 1989; Reich and

24   Lassoie, 1984). During moderately high O_3 exposure in a drought year, canopy transpiration was

25   greater for tulip poplar than on adjacent days with lower O_3 exposure. This could affect canopy

26   transpiration and landscape level water use (McLaughlin et al., 2004). Oxidant exposure (O_3 and

27   NO_x) may decrease the ability of exposed plants to close stomata at night (Grulke et al., 2004),

28   thus increasing water loss from the landscape. Ecosystem models should aid in interpreting

29   O_3-exposure effects at the landscape level.

30

9.7.4 Biotic Condition

9.7.4.1 Ecosystems and Communities

The SAB framework described by Young and Sanzone (2000) (Figure 9-20) identifies community extent, community composition, trophic structure, community dynamics, and physical structure as EEA's 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 sensitivity of the pines to O_3. 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

1   Laven 1999). In their projections, the population of *Pinus ponderosa* nearly disappears in all

2   tree age classes, and the community is dominated by *Quercus kelloggii* in all tree age classes,

3   followed by *Calocedrus decurrens* and *Pinus lambertiana* by the year 2074. Their projections

4   do not account for potential changes in genetic structure of the more O_3-sensitive species.

5   In the Valley of Mexico, the closed forest structure changed to a woodland from high

6   pollutant exposure (Miller et al., 2002). Cryptogamic community diversity also significantly

7   declined in response to prolonged, extreme O_3 exposure (Zambrano et al., 2002. Together,

8   these two examples illustrate the potential for shifts in community composition in response to

9   O_3 stress.

10

11   **TROPHIC STRUCTURE**

12   *Above-Ground Structural*

13   One of the first reports of trophic level interactions in natural communities was the

14   O_3-induced predisposition of *Pinus ponderosa* to attack by bark beetles (Stark et al., 1968; Cobb

15   et al., 1968; Stark and Cobb, 1969). Trees exposed to oxidant injury had lower resin production,

16   flow, and exudation pressure. Also, several attributes associated with tree defense against beetle

17   attack were compromised by oxidant exposure including sapwood and phloem moisture content

18   and phloem thickness (Pronos et al., 1999). Another trophic level has been implicated, in that O_3

19   injured ponderosa pine had the same rate of bark beetle infection, but healthy trees had greater

20   numbers of bark beetle predators and parasitoids (Dahlsten et al., 1997). This suggests that O_3

21   damage rendered the pines inhospitable for the natural enemies of the bark beetles. Similar

22   findings were presented by Percy et al. (2002) for aphids whose abundance was increased in

23   young *Populus tremuloides* stands exposed to elevated O_3. In that study, the levels of natural

24   enemies of alphids (ladybirds, lacewings, spiders and parasitoids) were significantly decreased

25   under elevated O_3.

26

27   *Below-Ground*

28   Processing of plant-derived carbon compounds by soil organisms comprising the soil food

29   web is a fundamental property of a functional and stable below-ground ecosystem (de Ruiter

30   et al., 1998; Wolters, 1998). Soil food web organisms are responsible for recycling nutrients and

31   for development of soil properties such as porosity, aggregate structure, water-holding capacity

1   and cation exchange capacity. A shift in food web species diversity or functional complexity in

2   response to O_3 stress may alter ecosystem processes.

3   Evidence that soil organisms are affected by O_3 indicates the potential for changes in soil

4   food web structure and function. Since O_3 does not penetrate the soil beyond a few centimeters,

5   the proposed mechanism by which O_3 alters soil biota is through a change in carbon input to

6   soils (Andersen, 2003). Ozone can alter C inputs to soil and hence soil processes through four

7   different pathways: (1) leaf-litter quality and quantity (see Material Cycling, below), (2) carbon

8   allocation to roots (see Physiological Status, below), (3) interactions among root symbionts, and

9   (4) rhizodeposition. The complex nature of the effects of O_3 on trophic interactions and food

10 webs calls for additional basic research and modeling.

11   There have been no comprehensive studies on the effects of O_3 on structural components of

12 soil food webs, however studies have shown that O_3 affects free-living soil organisms of food

13 webs. In the few cases where soil microbial communities have been examined, O_3 has led to

14 changes in bacterial and fungal biomass, and in some cases changes in soil enzyme activity.

15 Phillips et al. (2002) examined the effects of elevated CO_2 and O_3 on C flow through

16 heterotrophic microbial communities in soils collected from a FACE study in Wisconsin. Ozone

17 decreased abundance of fungal phospholipid fatty acids in aspen and birch-aspen plots but had

18 few other direct effects on measured soil parameters. The greatest effect of O_3 was to eliminate

19 significant increases in microbial respiration resulting from elevated CO_2, suggesting an

20 important role for O_3 in altering C flow through soils. Shafer (1988) found that O_3 tended to

21 increase the number of fungal propagules and bacteria exhibiting phosphatase activity in the

22 rhizosphere of sorghum. Ozone in combination with simulated acid rain stimulated soil

23 arylsufatase activity (Reddy et al., 1991). The response was observed at low concentrations, but

24 was reversed at high concentrations, suggesting a threshold level of O_3, possibly involving

25 different mechanisms. Ozone significantly decreased soil microbial biomass in the fall after one

26 season of exposure in a wheat (*Triticum aestivum*) and soybean (*Glycine max*) system (Islam

27 et al., 2000). Other studies have shown shifts in microbial and fungal biomass in response to O_3

28 stress, but responses were variable (Scagel and Andersen, 1997; Yoshida et al., 2001).

29   Decreased allocation to roots associated with O_3 exposure alters N fixation in legumes and

30 actinorrhizal species. Ozone exposure was found to decrease nodulation in a number of species

31 (Manning et al., 1971; Tingey and Blum, 1973). In alder (*Alnus serrulata*), host root cells of

1   nodules showed cytoplasmic breakdown and lacked organelles when seedlings were exposed to

2   27 d of O_3 (Greitner and Winner, 1989).

3   Ozone has been shown to affect mycorrhizal colonization (Ho and Trappe, 1981; McCool

4   et al., 1982; Simmons and Kelly 1989; Adams and O'Neill, 1991; Edwards and Kelly 1992;

5   Smith and Read, 1997). Although short-term in nature, several studies have found enhanced

6   mycorrhizal short-root formation under O_3 stress. White pine (*Pinus strobus*) (Stroo et al.,

7   1988), Norway spruce (Rantanen et al., 1994), Northern red oak (*Quercus rubra*) (Reich et al,

8   1985), Douglas-fir (*Pseudotsuga menziesii*) (Gorissen et al., 1991), European silver-fir (*Abies*

9   *alba*) (Wollmer and Kottke, 1990), and Scots pine (*P. sylvestris*) (Kasurinen et al., 1999) all

10 showed some increase in mycorrhizal presence when exposed to O_3. Others have shown

11 minimal or no effects of O_3 on mycorrhizas (Mahoney et al., 1985; Meier et al., 1990;

12 Kainulainen et al., 2000). Stroo et al. (1988) found that percent infection increased from 0.02 to

13 0.06 ppm O_3, then decreased from 0.06 to 0.14 ppm; the total number of short roots were

14 unaffected, however. In cases where stimulation was observed, the response was often noted

15 shortly after initiation of exposure, often at relatively low concentrations. Good examples of this

16 transitory response can be found in results with Norway spruce and Scots pine (Ratanen et al.,

17 1994; Kasurinen et al., 1999). In these studies, O_3 increased mycorrhizal short roots initially but

18 differences were not evident by the end of the experiment.

19   There is evidence which suggests that decreased below-ground allocation associated with

20 O_3 stress alters mycorrhizal host-symbiont compatibility. Edwards and Kelly (1992) found a

21 shift in fungal morphotypes present on loblolly pine roots, even though the number of

22 mycorrhizal short roots per gram fine root was not significantly affected by O_3. Qiu et al. (1993)

23 found increased numbers of morphotypes present on O_3-sensitive loblolly pine seedlings exposed

24 to O_3. Roth and Fahey (1998) found an interaction between O_3 and acid precipitation treatments

25 on the composition of fungi forming ectomycorrhizae on red spruce saplings, possibly driven by

26 nutrient availability. Carbohydrate requirements vary among fungal species (Bidartondo et al.,

27 2001), and O_3 may affect species composition by altering carbohydrate availability in roots.

28 A shift in species dominance could lead to a change in successional patterns of mycorrhizal

29 communities.

30   In the few studies that examined root exudation in response to O_3 exposure, O_3 was found

31 to alter the quantity and quality of root exudates. McCool and Menge (1983) found a significant

decrease in exudation of amino acids in tomato (*L. esculentum*) exposed to 300 ppb O_3. McCrady and Andersen (2000) observed increased root exudation in nonmycorrhizal wheat (Triticum aestivum). No apparent change in root exudation was found in labeling studies of ECM ponderosa pine (Andersen and Rygiewicz, 1995). Inconsistency in the literature probably results from species differences and experimental protocols, however these examples illustrate the potential effects of O_3 on rhizosphere carbon flux.

Decreased carbon 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; Scagel and Andersen, 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, 1995).

COMMUNITY DYNAMICS, PHYSICAL STRUCTURE

One of the best-documented examples of change in long-term forest community dynamics of dominant overstory trees occurred in the San Bernardino Mountains between 1968 and 1974 (reported in Miller, 1973; 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 sensitive species (Miller et al., 1983), ponderosa and Jeffrey pine, had the lowest increase in basal area/ha (76 and 62%, respectively). These two species represented 72%

1   of the basal area/ha of all stands inventoried. Ponderosa pine had the greatest mortality rate of

2   all canopy species inventoried (46%), followed by white fir and black oak (35% and 33%),

3   Jeffrey pine (29%) and incense cedar and sugar pine (both 7%). In moist sites (at the western

4   end of the San Bernardino Mountains), there was significant recruitment of incense cedar, white

5   fir, and sugar pine. Only one study directly attributed tree mortality to O_3 exposure: it

6   accounted for 7% of mortality in the Sierra and Sequoia National Forests (Carroll et al., 2003).

7   Species diversity in the understory can be quite large, making studies of O_3 effects on

8   understory community dynamics very challenging. However, there have been some attempts to

9   quantify understory responses, ranging from describing relative sensitivity to their visible

10 symptoms (Treshow and Stewart, 1973; Temple, 1999) to very complex measures of community

11 structure and composition (Westman, 1979, 1981). The lowest percentage cover and lowest

12 species diversity in California coastal sage scrub was correlated with the highest O_3 exposures as

13 estimated by extrapolation from the closest air monitoring stations (Westman 1979). The

14 understory also has the potential to influence responses to O_3 of dominant keystone species, as

15 has been shown in controlled experiments with both *ponderosa pine* (Andersen et al., 2001) and

16 *loblloly pines* (Barbo et al., 2002). Barbo et al., (1998) exposed an early successional forest

17 community to ambient air, charcoal-filtered air, non-filtered air, and 2× ambient in the

18 Shenandoah National Park. They found changes in species performance, canopy structure,

19 species richness and diversity index consistent with the view that O_3 can induce a shift in

20 vegetation dominance and community structure.

21   There have been few studies evaluating the effect of O_3 exposure on the physical structure

22 of natural ecosystems. Despite an extensive array of allometric equations for conifers in the

23 west on United States (Ter-Mikaelian and Korzukhin, 1997), none appear to predict individual

24 tree shape in a site of moderate O_3 exposure, suggesting that O_3 may effect allometry (Grulke

25 et al., 2003a). Canopy structural changes are also implied by the measure of canopy

26 transparency used in the FHM assessment. The loss of epiphytic lichens within the canopy is a

27 clear example of plant community structural change occurring along an O_3 gradient (Nash and

28 Sigal, 1999; Zambrano et al., 2002).

29   As of yet, there have been no comprehensive studies on the effects of O_3 on structural or

30 functional components below-ground (Andersen, 2003). Phillips et al. (2002) found evidence

31 for changes in the bacterial and fungal biomass below *Populus tremuloides* and

1   *P. tremuloides*/*Betula papyrifera* stands exposed to elevated O_3. Subsequent study showed that

2   O_3 exposure decreased cellobiohydrolase activity in the soil microorganisms, driving the change

3   in the microbial community (Larson et al., 2002).

4

5   **9.7.4.2   Species and Populations**

6   Ozone can affect species and populations of species comprising ecosystems through

7   changes in population size, genetic diversity, population structure and/or dynamics, and habitat

8   suitability (Young and Sanzone, 2002). For example, if individuals of a species are lost due to

9   O_3 exposure, population size declines. Often very young (e.g., conifer seedlings, see Section

10 9.7.4.3 below) and old individuals differ in their sensitivity, so that population structure also will

11 be altered by O_3 exposure. If resource allocation to reproductive output is altered by O_3

12 exposure, population dynamics will be altered. Communities dominated by O_3 sensitive species

13 in the canopy or understory may be altered sufficiently for the habitat to become unsuitable for

14 other species. Genetic selection acts on the individual plant, which represents a certain

15 proportion of the populations' genetic variation. If an O_3 sensitive individual succumbs through

16 multiple stresses, including O_3 stress, the genetic variation represented in the population

17 generally declines, unless sensitive individuals have low inherent genetic variability (e.g.,

18 Staszak et al., 2004).

19   While the concept of natural selection induced by O_3 exposure and related changes in

20 natural plant communities has been around for a long time (Dunn, 1959; Miller et al., 1972), the

21 concept of evolution of O_3 tolerance is still not widely accepted. The unequivocal demonstration

22 that considerable genetic variation in O_3 resistance exists within and between plant populations,

23 and that ambient levels of O_3 may differentially affect fitness-related traits (i.e., growth, survival,

24 and fecundity), suggests that O_3 may potentially drive the natural selection for resistant

25 individuals. Dunn (1959) presented circumstantial evidence that ambient O_3 in the Los Angeles

26 area was high enough to drive the selection of O_3 resistant *Lupinus bicolor* genotypes. Since

27 Dunn's (1959) research on O_3-induced population changes, researchers have demonstrated

28 differences in O_3 tolerance among other plant populations. In the devastating forest decline

29 southeast of Mexico City, the remaining trees (primarily *Abies religiosa* in the "cemetery

30 forests") appear to be less affected by foliar injury than trees that were lost (Alvarado-Rosales

31 and Hernandez-Tejeda, 2002), despite continued, high O_3 exposures (Bravo-Alvarez and

1   Torres-Jardon, 2002). However, even the most convincing work in this field (Berrang et al.,

2   1986, 1989, 1991), with *Populus tremuloides*, where a strong correlation between visible foliar

3   injury after O_3 exposure and maximum O_3 concentration at the origin of the population was

4   shown (Berrang et al., 1991), a change in gene frequency at any one site over time has not yet

5   been demonstrated (Bell et al., 1991; Reiling and Davison, 1992b). Furthermore, the selection

6   intensity of O_3 has been questioned (Bell et al., 1991; Taylor and Pitelka, 1992; Taylor et al.,

7   1994) and the emergence of O_3 exposure since the 1950's as an environmental stressor may not

8   have been long enough to affect tree populations with long generation times (Barrett and Bush,

9   1991).

10   The loss of O_3-sensitive individuals results in natural selection favoring O_3-tolerant species

11   (Bradshaw and McNeilly, 1991). Increased levels of mortality of O_3-sensitive individuals have

12   occurred for *Pinus jeffreyi* and *P. ponderosa* exposed to ambient O_3 along the western slope of

13   the Sierra Nevadas (Peterson et al., 1987; Miller et al., 1998), for *Pinus strobus* exposed to

14   ambient O_3 in southern Wisconsin (Karnosky, 1981), and for *P. ponderosa* in the San Bernardino

15   Mountains (Carroll et al., 2003). In these examples, individuals that consistently had greater

16   foliar injury and lower needle retention were lost in repeated surveys. Ozone-induced loss of all

17   individuals except the most tolerant and breeding among the surviving individuals to yield more

18   more tolerant populations has not yet been demonstrated for plants exposed to O_3, except for the

19   relatively short term (2 years) adaptation exhibited in *Trifolium repens* (Heagle et al., 1991) and

20   *Plantago major* (Davison and Reiling, 1995). Heagle et al. (1991) were able to show the

21   adaptation of a *Trifolium repens* population to elevated O_3 in just two growing seasons.

22   Similarly, Davison and Reiling (1995) compared the O_3 resistance of *P. major* populations

23   grown from seed collected from the same sites over a period of increasing O_3. The two

24   independent populations studied exhibited increased O_3 resistance, consistent with the idea of

25   selection for O_3 tolerance. Using random amplified polymorphic DNA primers, this team also

26   showed that the later populations are subsets of the earlier ones, consistent with in-situ evolution

27   rather than with catastrophic loss and replacement of the populations (Wolff et al., 2000). The

28   problem is determining whether spatial patterns in O_3 resistance and changes in time are casually

29   related to O_3, because there were very strong correlations with other factors (Reiling and

30   Davison, 1992a; Davison and Barnes, 1998). The potential for evolution of O_3 resistance has

31   been clearly demonstrated by Whitfield et al. (1997) in their study of O_3 selection of common

1 plantain (*Plantago major L.*), where they showed that within a matter of a few generations, it

2 was possible to increase O_3 resistance in an initially O_3-sensitive population. Wild radish

3 (*Raphanus sativus L.*) developed O_3 resistance after only one generation of exposure to O_3

4 (Gillespie and Winner, 1989).

5   A third independent line of research suggesting O_3 may be affecting the genetic diversity of

6 wild plant populations was presented by Paludan-Müller et al. (1999) who showed that northwest

7 European provenances of European beech (*Fagus sylvatica L.*) were more sensitive to O_3 than

8 were southeast European provenances which had experienced higher O_3 levels. Recent research

9 on the genetic structure of 50-year-old *ponderosa pines* in the San Bernardino Mountains

10 suggests that distinct differences in frequency of some alleles and genotypes occurred, with the

11 O_3-tolerant trees being more heterozygous (Staszak et al., 2004). While both of these studies

12 were only correlational, they are consistent with previous studies of this type suggesting

13 O_3-induced population changes. Again, other environmental stressors besides O_3 exposure could

14 have been involved in effecting change within these populations.

15   Natural selection for O_3 tolerance can also be facilitated by reductions in fitness related to

16 lower seed yields of O_3-sensitive species or individuals. The impacts of O_3 on reproductive

17 development, recently reviewed by Black et al. (2000) can occur by influencing: (1) age of

18 flowering, particularly in long-lived trees that often have long juvenile periods of early growth

19 without flower and seed production; (2) flower bud initiation and development; (3) pollen

20 germination and pollen tube growth; and (4) seed, fruit, or cone yields and seed quality

21 (Table 9-24). In addition, vegetatively propagated species can have lower numbers of

22 propagules under elevated O_3 conditions (Table 9-24).

23   Several studies suggest that reproductive structures are clearly sensitive to O_3 and that O_3

24 can affect fitness of plants by affecting either the sporophytic or gametic generations. Decreased

25 numbers of flower spikes and seed capsules per plant were found for plantain growing under

26 elevated O_3 (Reiling and Davison, 1992b; Pearson et al., 1996; Lyons and Barnes, 1998).

27 Similar responses were seen for *Brassica campestris L.* plants exposed to a single dose of

28 100 ppb O_3 for 6 h. Stewart et al. (1996) and Bosac et al. (1998) reported an increase of flower

29 bud abortion for oilseed rape (*Brassica napus L.*) similarly exposed to a short duration of

30 elevated O_3. Floral initiation period can be delayed in O_3-sensitive plants, as was described for

31 dogbane (*Apocynum androsaemifolium*) grown under ambient O_3 in the eastern United States.

1   In one of the few comparisons of whole plant O_3 sensitivities with that of male gametophytes,

2   Hormaza et al. (1996) found a high correlation of relative O_3 sensitivity of pollen tube elongation

3   with that of O_3 effects on net photosynthesis and relative growth rates for 6 species of fruit trees.

4   Clearly, the concept of O_3-induced genetic change is an area that needs additional research

5   attention. Repeated collections over time from wild populations receiving high O_3 exposures to

6   examine population responses and relative sensitivity changes, the sampling of genetic diversity

7   along known O_3 gradients, and the use of modern biotechnological approaches to characterize

8   and quantify genetic diversity are useful approaches to test for O_3-induced impacts on diversity

9   in natural ecosystems.

10

11   **9.7.4.3 Organism Condition**

12   **PHYSIOLOGICAL STATUS**

13   The generalized effects of O_3 exposure on plants are well known, and have been reviewed

14   from several viewpoints over the last decade (De Kok and Tausz, 2001; Heath and Taylor, 1997;

15   Pell et al., 1997; Schraudner et al., 1997; Matyssek et al., 1995; Darrall 1989; Reich, 1987; U.S.

16   Environmental Protection Agency, 1996). The topic of individual species response and

17   modification of response by other factors has been addressed thoroughly in Sections 9.4.3 and

18   9.4.4 of this chapter. Here, physiological changes in response to O_3 that have been hypothesized

19   to lead to changes in ecosystem structure or function are highlighted.

20

21   *Above-Ground Responses*

22   The first critical step leading to O_3 response is uptake and movement of O_3 by the leaves,

23   leading to changes in C and nutrient relations that are thought to alter plant growth and

24   competiveness (see Section 9.3). Ozone enters leaves through stomates, reacts with cell walls or

25   membranes, and starts a series of adverse reactions. Cuticular uptake of O_3 is believed to be

26   negligible (Kerstiens and Lendzian, 1989; Coe et al., 1995). Once inside the leaf, O_3 and its

27   byproducts lead to membrane disruption, chlorophyll breakdown, and decreased Rubisco levels

28   (Schweizer and Arndt, 1990). In turn, photosynthesis is decreased, as is stomatal conductance

29   (Weber et al., 1993). Also, O_3 often leads to increased maintenance respiration, decreased foliar

30   nutrient content, and imbalances in tissue nutrient content and retention. When photosynthetic

31   pigments have been damaged, the pigment must be fully broken down (and/or new N and Mg

1   must be taken up and transported to the leaf) for the pigment to be regenerated (Bjorkman and

2   Demmig-Adams, 1995). Ozone exposure alters within-plant priorities for resources: less C is

3   available for allocation to roots and spring regrowth, and less foliar biomass is retained. At the

4   whole-organism level, O_3 exposure decreases root mass (Grulke et al., 1998) and radial bole

5   growth (Peterson et al., 1991; Muzika et al., 2002) with little impact on height growth. Visible

6   symptoms of O_3 injury vary between species and genotypes but often include upper leaf surface

7   stipple, chlorotic mottle, or large bifacial blotches of necrotic tissue. Premature senescence is

8   typical of almost all O_3-induced foliar damage. All of these changes can alter the plant's ability

9   to function in a broader ecosystem context.

10   The underlying mechanisms of O_3-injury response in conifers, broadleaf deciduous trees,

11   and herbaceous species are assumed to be similar. However, several differences in long-lived

12   species are important at the ecosystem level. Most of the research on O_3 effects has been

13   conducted on herbaceous species (i.e., crops). Although a number of native herbaceous species

14   have been identified as O_3-sensitive. there are no published physiological studies on the effect of

15   O_3 exposure on herbaceous or shrub species in situ. In natural ecosystems, the majority of

16   species are not annuals, unless the system is highly disturbed. Nonetheless, response of crop

17   species to elevated O_3 may be used as an analog for native annual response: phenological

18   staging is accelerated (soybeans; Booker et al., 2004), thus "avoiding" additional O_3 exposure.

19   Conifers have roughly half the stomatal conductance of deciduous broadleaf trees (Reich,

20   1987), leading to proportionally less O_3 uptake at the same O_3 exposure level. Yet, except for

21   species of larch, individual conifer needles are longer-lived and active over a greater portion of

22   the year. Therefore, needle longevity can also work against the tree by increasing cumulative O_3

23   exposure and exposure to other stressors. Increased needle longevity is not always a

24   disadvantage, for example, conifers are physiologically active in early spring and late fall, during

25   times of lower oxidant concentrations. These periods can contribute significantly to a net

26   positive annual C balance and from the standpoint of nutrient storage, are important in reparation

27   responses to pollutants. Patterson and Rundel (1995) reported that Jeffrey pine had significant

28   stomatal opening (one third that of a typical summer day) in mid-winter with snow on the

29   ground. At least pole-sized and larger trees can mitigate reductions in C acquisition due to

30   oxidant exposure in the summer with C assimilation on favorable days in the winter. The

31   interaction of environmental factors, plant phenology (the timing of growth events; birth and

1　mortality of plant parts), physiological status (nutritional or moisture status; dormant or active

2　growth within the year), and tree age (interannual differences in resource acquisition and

3　allocation) all contribute to the complexity of long-lived species (and hence ecosystem) response

4　to O_3 exposure.

5　　　　One widely observed response to O_3 exposure is premature leaf loss. As noted above,

6　premature leaf loss may reduce O_3 uptake during high-O_3 years, but it has several negative

7　consequences. Early leaf loss results in reduced C uptake through photosynthesis. Premature

8　needle loss also results in less N retranslocation compared to normally senescing leaves, which

9　reduces whole plant N balance (Fenn and Dunn, 1989) and carbohydrate availability for

10　overwinter storage (Grulke et al., 2001). Because of such effects accumulated over several years

11　of O_3 exposure, subsequent-year C and N reserves can be affected (Andersen et al., 1991).

12　　　　Conversely, a series of drought years can decrease O_3 uptake, as well as reduce C and

13　nutrient acquisition, altering resource allocation to defenses (e.g., antioxidants) (Grulke et al.,

14　2003b) (or resins) against insect infestation, rendering the tree more susceptible to O_3 injury.

15　Conifers have thicker cuticles than either broadleaf deciduous or herbaceous species. Continued

16　O_3 exposure may compromise cuticular integrity (Percy et al., 1994). Once cuticular integrity is

17　breached, individual leaves (needles) are likely to be excised, thus contributing generally to

18　defoliation and reduced C acquisition.

19　　　　With the exception of the extensive research conducted on mature tree response to O_3

20　exposure in California forests (Peterson et al., 1987, 1991, 1995, Arbaugh et al., 1998; Grulke

21　1999; Grulke and Balduman 1999; Grulke et al., 1996, 1998, 2001, 2002b, 2003a,b, 2004;

22　Weiser et al., 2002), the vast majority of studies of O_3 effects on forest trees have been

23　conducted on young seedlings (Chappelka and Samuelson, 1998) and little is known about

24　acclimation to O_3 (Skärby et al., 1998). Chamber exposure studies can be used to document

25　foliar symptoms and develop response variables for the whole plant. These response variables

26　can then be field tested on mature trees using correlative analyses (e.g., Grulke and Lee, 1997;

27　Grulke et al., 2003b). Without the initial work in chamber exposure studies, field responses to

28　O_3 exposure would be difficult to verify and distinguish from other concurrent stressors.

29　　　　Predicting mature tree responses to O_3 solely from seedling response studies is complex,

30　because seedlings or saplings do not necessarily respond to O_3 in the same way as mature trees

31　(Norby et al., 1999; Karnosky, 2003). Ozone has been found to have stronger effects on leaf

1   function in younger rather than older trees (Kolb and Matyssek, 2001). Each component

2   physiological attribute "matures" at a different rate. Gas exchange patterns differ between

3   seedlings and mature trees. For example, leaf respiration of juvenile ponderosa pine was greater

4   than that of mature trees (Momen et al., 1996). In the conifers tested, the highest gas exchange

5   rates (and by inference stomatal uptake of O_3) are found in seedlings (e.g., in scions of red

6   spruce, Rebbeck and Jensen, 1993; giant sequoia, Grulke et al., 1994; ponderosa pine, Grulke

7   and Retzlaff, 2001; and Norway spruce, Wieser et al., 2002). Patterns of biomass (Grulke and

8   Balduman, 1999) and carbohydrate allocation (Grulke et al., 2001) differs between immature and

9   mature trees. Pole-sized trees had greater reduction in root, foliar, and bole carbohydrate

10   concentrations than did old growth trees. Antioxidant defenses vary with both tree age and

11   needle age (Tegisher et al., 2002). Based on all attributes measured in both ponderosa pine and

12   giant sequoia, the youngest tree age considered representative of mature trees was 20 years old

13   (Grulke et al., 1996; Grulke and Retzlaff, 2001). In some broadleaf deciduous tree species,

14   seedlings are more conservative, and mature trees have greater gas exchange rates, as is the case

15   for *Quercus robur* (Edwards et al., 1994; Kelting et al., 1995; Samuelson and Kelly, 1996, 1997)

16   and *Fagus sylvatica* (Braun et al., 1999). In another broadleaf deciduous tree species (cherry,

17   *Prunus serotina*), gas-exchange rates of seedlings were faster, but total O_3 flux to leaves of

18   seedlings was lower than that of mature trees due to differences in leaf ontogeny (Frederickson

19   et al., 1995, 1996).

20   Nitrogen deposition modifies the effects of oxidant exposure through several offsetting

21   physiological mechanisms (see Section 9.4.4). Nitrogen deposition, in wet or dry particulate

22   form, ultimately increases site fertility, but increased soil N availability decreases C allocation to

23   roots, further exacerbating the effects of O_3 exposure on roots (Grulke et al., 1998). Increased N

24   availability also increases foliage turnover: fewer needle age classes are retained (Gower et al.,

25   1993). Therefore, the combination of both increased N and O_3 exposure increases foliar

26   turnover. Finally, N deposition and increased plant N nutrition can increase stomatal

27   conductance, leading to increased O_3 uptake. Alternatively, increased N counteracts the effect of

28   O_3 on photosynthesis by increasing photosynthetic pigments and enzymes. Nitrogen deposition

29   may mitigate the degree of foliar injury from oxidant pollution via higher available N for

30   reparation of photosynthetic pigments. Nitrogen amendments also modify the antioxidant

31   defense system in complex ways (Polle, 1998).

1   Attributes of O_3 injury to trees (foliar injury, needle retention, and canopy transparency), as

2   well as presence of pathogens and insect infestation, are routinely inventoried in established

3   sample plots distributed on Federal lands across the United States (Forest Health Protection,

4   USDA Forest Service). Foliar injury to several widespread, herbaceous species nationally

5   recognized as sensitive (bioindicators) is also assessed (NPS, 2003). This assessment is part of a

6   larger assessment of forest tree growth and dynamics (the Forest Inventory and Analysis

7   Program; Smith, 2002; Smith et al., 2003). Risk of O_3 injury is then estimated for the dominant

8   forest tree species in the sample plots. For example, 12% of sampled black cherry (*Prunus*

9   *serotina*), 15% of loblolly pine (*Pinus taeda*), and 24% of sweetgum (*Liquidambar styraciflua*)

10   were found to be in the highest risk category in the northeast and mid-Atlantic states (Coulston

11   et al., 2003). In the Carpathian Mountains, 12 to 13% of all trees (broadleaf and coniferous)

12   have greater than 26% crown defoliation (Badea et al., 2002). In general, broadleaves (primarily

13   beech) trees were less affected (8 to 45%) than spruce (up to 37%) and fir (up to 50%)

14   (Grodzinska et al., 2002). Ozone injury was directly correlated with cumulative O_3 exposure in

15   the Sierra Nevada Mountains (Arbaugh et al., 1998); with the best correlation being found across

16   sites where > 90% of the trees had O_3 injury. Although direct links of visible foliar symptoms

17   induced by O_3 to adverse effects on biomass are not always found, visible foliar symptoms have

18   been linked to decreased vegetative growth (Peterson et al., 1987; Karnosky et al., 1996; Somers

19   et al., 1998), as well as reproductive function (Black et al., 2000; Chappelka, 2002).

20   Foliar O_3 injury has also been associated with adverse effects on competitive ability and

21   survival in forest communities (Karnosky, 1981; McDonald et al., 2002; Karnosky et al., 2003b).

22   Competition can alter organism condition and affect susceptibility to O_3. Ponderosa pine

23   seedlings were more susceptible to O_3, as determined by decreased plant biomass, when grown

24   in competition with blue wild-rye grass (Andersen et al. 2001). Similarly, the magnitude of O_3

25   effects on height and diameter growth depended on the competitive status of *Populus*

26   *tremuloides* trees (McDonald et al., 2002). These studies show the importance of including

27   competition as a concurrent stressor in assessing whole plant responses to O_3. The age of the

28   community ("time since disturbance") may also affect the ability of individuals to effectively

29   respond to O_3. Unfortunately, the vast majority of O_3 studies have been conducted on

30   open-grown plants, often grown in pots where competition is absent both above and below

31   ground.

1   Clearly, age-dependent O_3 responsiveness and juvenile-mature correlations remain

2   important research questions in attempting to scale up to ecosystem level responses. Patterns of

3   allocation between root, stem, and leaf differ between immature and mature trees. Tree

4   architecture varies with tree age, and leaf area distribution in space and time may change in

5   response to elevated O_3. All of these factors influence gas exchange in the canopy.

6   Furthermore, there may be few generalities that can be made from seedling to mature tree

7   response to O_3 within a plant functional group (Norby et al., 1999; Karnosky, 2003).

8   Consequently, modeling ecosystem response is limited to either dealing with mono-specific

9   plantations or assigning average responses to a mix of species.

10

11   *Below-Ground Responses*

12   The effect of O_3 on the soil ecosystem is thought to occur through physiological changes in

13   the root and interactions with soil organisms (Andersen, 2003). Comparatively little is known

14   about how changes in root growth and metabolism are translated through the soil food web,

15   resulting in changes in soil and hence, ecosystem processes. An overview of physiological

16   changes likely to lead to changes at the ecosystem level is provided below.

17   Ozone stress decreases carbon allocation to roots (Manning et al., 1971; McLaughlin and

18   McConathy, 1983; McCool and Menge, 1983; Cooley and Manning, 1987; Gorissen and van

19   Veen, 1988; Spence et al., 1990; Gorissen et al., 1994; Rennenberg et al., 1996; U.S.

20   Environmental Protection Agency, 1996). Since roots are often dependent on current

21   photosynthate for their structural development (van den Driessche, 1978; Ritchie and Dunlap,

22   1980; Marshall and Waring, 1985; van den Driessche, 1991), C-limiting stresses such as O_3 can

23   have rapid and significant effects on root growth. In many cases, decreased allocation to roots in

24   response to O_3 occurs quickly, with reductions in root growth occurring within one growing

25   season (Gorissen and van Veen, 1988; Spence et al., 1990; Gorissen et al., 1991; Andersen and

26   Rygiewicz, 1991, 1995; U.S. Environmental Protection Agency, 1996). Decreased C allocation

27   below ground is often associated with decreased root-shoot ratio, but observed responses in

28   root-shoot ratio are highly variable owing to several factors including intra- and interspecies

29   variation, culture conditions, and ontogenetic drift (Reich, 2002). Root-shoot ratio is a

30   point-in-time measurement that does not include C lost to exudation, respiration or turnover.

1   Therefore, biomass and ratios of biomass (such as root-shoot ratio) do not necessarily reveal

2   physiological changes in response to O_3 stress.

3   Decreased C acquisition leads to reduced carbohydrate levels and storage pools in

4   O_3-exposed plants (Tingey et al., 1976; Ito et al., 1985; Cooley and Manning, 1987; Rebbeck

5   et al., 1988; Gorissen et al., 1994; Andersen et al., 1997; McLaughlin et al., 1982). Although it

6   is difficult to quantify changes in the field, Grulke et al. (1998) found decreased medium and

7   fine root biomass with increased pollutant load across an O_3 gradient in southern California.

8   Coarse and fine root starch concentrations also were lowest in mature trees at the most polluted

9   site (Grulke et al., 2001). The effects of O_3 could not be completely separated from other known

10   stresses across the pollutant gradient, but it appeared that O_3 was an important factor in the

11   patterns observed.

12   Decreased storage pools can lead to carry-over effects on root growth that are compounded

13   over time. Decreased carbohydrate storage pools were associated with decreased root growth

14   during the spring following exposure to O_3, even in the absence of additional O_3 exposure

15   (Andersen et al., 1991, 1997). Decreased spring root growth was attributed to decreased stored

16   C reserves as well as to premature loss of older foliage age classes during the previous fall.

17   Aside from the loss of photosynthetic surface area associated with premature senescence, early

18   loss of foliage in the fall occurs when allocation to roots is at a maximum in many species

19   (Kozlowski and Pallardy, 1997). Older needle age classes preferentially allocate photosynthate

20   basipetally to stems and roots (Rangnekar et al., 1969; Gordon and Larson, 1970), and the loss of

21   older needles in the fall during allocation to root growth and storage, and in the spring during

22   periods of root growth, preferentially impacts roots and root processes.

23   Ozone has also been shown to affect root metabolism as evidenced by changes in root

24   respiration. Edwards (1991) found decreased root and soil CO_2 efflux during a 2-year exposure

25   of loblolly pine to O_3. Fine root respiration increased in mature red oak exposed to O_3, while

26   total soil CO_2 efflux increased in the spring and decreased in the summer and fall (Kelting et al.,

27   1995). The authors attributed increased root respiration to increased nutrient uptake in support

28   of increased demands in the shoot. Ozone decreased root system respiration in aspen after 12

29   weeks of exposure, but the decrease was closely associated with decreased root biomass and

30   probably not metabolic processes (Coleman et al., 1996). Whether other metabolic shifts occur

31   in the roots of plants exposed to O_3 needs to be examined.

1   Measurable effects on roots may occur before effects on shoots are observed because
2   shoots have immediate access to C for repair and compensation whereas roots must compete
3   with shoots for C. Mortensen (1998) found decreased root but not shoot growth in *Betula*
4   *pubescens* at O_3 exposures of 42 nMol mol-L (applied 12 h d^{-1}), whereas both root and shoot
5   growth were reduced at higher exposures. Chromosomal aberrations were found in root tips of
6   Norway spruce exposed to O_3, even in the absence of biochemical changes in needles (Wonisch
7   et al., 1998, 1999). Using relatively high O_3 concentrations (0.15 ppm O_3 6 h d^{-1}), Hofstra et al.
8   (1981) found metabolic changes in *Phaseolus vulgaris* root tips prior to the development of leaf
9   injury. Morphological changes in root tips occurred within 2 to 3 days, and metabolism declined
10   within 4 to 5 days of initiation of O_3 exposure.
11   Feedback signals from roots can influence the degree of O_3 response. Stolzy et al. (1964)
12   exposed tomato roots (*Lycopersicon esculentum*) to periods of anerobic conditions and followed
13   a change in leaf susceptibility to O_3. An exposure of roots to low oxygen conditions for 3 h did
14   not alter photosynthesis, but foliar damage was decreased when the roots were subsequently
15   exposed to O_3. In this case, a signal originating in the root appeared to alter leaf sensitivity to
16   O_3, the signal possibly being hydraulic in nature and leading to decreased O_3 uptake.
17
18   **SYMPTOMS OF DISEASE OR TRAUMA, SIGNS OF DISEASE**
19   Although insects and diseases are dynamic components of forest ecosystems, trees can be
20   especially susceptible to outbreaks due to the presence of multiple stressors such as drought and
21   pollutant exposure. Ozone can have direct effects on insect or disease organisms, indirect effects
22   on the insect or pathogen through changes to the host, and direct or indirect effects on natural
23   enemies of the insect or pathogen (Pronos et al., 1999). A full discussion of O_3 effects on insect
24   and pathogen interactions can be found in Section 9.4.
25   Although the multitude of interacting factors makes it difficult to identify causative factors
26   in the field, some recent examples suggest a role for O_3 in the timing or magnitude of disease
27   attacks in the field. After periods of drought stress (such as 1995 in central Europe), the
28   incidence of bark beetle (*Ips spp.*) appears to increase. In 1998 and 1999, the mean daily capture
29   of *Ips* was lowest in plots with low O_3 exposure; the converse was also true (Grodzki et al.,
30   2002). Elevation confounded the relationships, but the differences in *Ips* frequency in relation to
31   O_3 concentrations were highly significant at lower elevations. In the Valley of Mexico, a 1982

1   to 1983 drough was documented, but not described as precipitating a bark beetle attack in the

2   early 1980's. However, a link between air pollutant exposure and bark beetle attacks were

3   implicated, because attacked trees were already O_3-stressed at the time of the bark beetle attack

4   (Alvarado-Rosales and Hernandez-Tejeda, 2002).

5   An early study showed that oxidant exposure predisposed *ponderosa pine* to the root

6   pathogen *Fomes annosus* (James et al., 1980). Both root diseases (Pronos et al., 1999) and O_3

7   exposure (Grulke and Balduman, 1999) can each reduce root biomass, leading to increased

8   drought stress, insect attack, and subsequent windthrow or death. Trees may take several years

9   to die, and the patterns of precipitation and annual total rainfall interact to drive the level of

10   drought stress experienced by the tree (Pronos et al., 1999). Additional research is necessary to

11   fully understand the complex interactions occurring between O_3 stress and other biotic stresses.

12

13   ## 9.7.5 Ecosystem, Chemical, and Physical Characteristics (water, soil)

14   ### 9.7.5.1 Nutrient Concentrations, Trace Inorganic and Organic Chemicals

15   Ozone exposure reduces the nutritional content of tissues, as well as causing elemental

16   imbalances. Foliar nutrient content may be too high (toxic) or too low (deficient), but the

17   relative amounts and ratios among all nutrients can also result in imbalances.

18   Although N deposition and foliar N content increased with O_3 exposure in the San

19   Bernardino Mountains, K, Mg, Fe, and Al were all also higher in ponderosa pine at sites more

20   exposed to air pollution (Poth and Fenn, 1998). Trees with greater foliar injury (due to O_3

21   exposure) had higher current year needle concentrations of P, K, Zn, and Fe than trees at the

22   same site that were less injured. In drought-stressed ponderosa pine with O_3 exposure, foliar N

23   was also elevated and retained in the remaining needles (Temple et al., 1992). At a relatively

24   clean site in the eastern San Bernardino Mountains, N, P, and K were efficiently readsorbed, but

25   P remaining in the foliage was relatively high compared to defined thresholds. The fact that

26   other elements were modified besides the N being deposited emphasizes the degree of chemical

27   imbalance in the tissue. Foliar micronutrients were within the normal ranges reported for

28   ponderosa pine (Poth and Fenn, 1998; Powers, 1981). Because both N deposition and O_3

29   exposure reduce root biomass, it was unlikely that the foliar nutrient content was higher due to

30   greater uptake. Instead, it appaears that retranslocation from senescing tissue was responsible.

31   Across a pollution gradient in the Carpathian Mountains in eastern Europe, only S/N (expected

1   due to high S deposition) and Fe/Mn ratios were out of balance relative to established norms.

2   The S was relatively high due to SO_2 deposition, and the Fe was relatively high due to smelter

3   plumes. No imbalances could be directly attributed to O_3 exposure (Mankovska et al., 2004).

4

5   **9.7.6 Ecological Processes**

6   **9.7.6.1 Energy Flow**

7   All green plants generate and use energy-containing C compounds through the processes of

8   photosynthesis and respiration. Whole-plant C uptake is dependent on photosynthesis rates, leaf

9   area, and leaf phenology. The effects of O_3 at the site of action in the leaf are discussed in

10   Section 9.3. Here, the main focus is on whole-plant carbon dynamics resulting from changes in

11   C acquisition or use under O_3 stress.

12   In natural ecosystems, O_3 has been shown to depress photosynthesis in sensitive tree

13   species including *Pinus ponderosa* (Miller et al., 1969; Weber et al., 1993; Takemoto et al.,

14   1997; Grulke et al., 2002b) and *Populus tremuloides* (Coleman et al., 1995a; Yun and Laurence,

15   1999; Noormets et al., 2001a,b; Sharma et al., 2003). In a study of mature Jeffrey pine, trees in

16   mesic microsites had greater O_3 uptake over the growing season in comparison to trees in xeric

17   microsites (Grulke et al., 2003a) and greater O_3 uptake was correlated with lower mid canopy

18   needle retention, lower branch diameters, and lower foliar N content (Grulke et al., 2003a).

19   Chamber studies have also shown negative effects of O_3 on tree seedling canopy structure

20   (Dickson et al., 2001) and leaf area (Neufeld et al., 1995; Wiltshire et al., 1994). It is well

21   known from chamber and field studies that O_3 exposure is correlated with lower foliar retention

22   (Miller et al., 1963; 1972; Karnosky et al., 1996; Grulke and Lee, 1997; Pell et al., 1999; Topa

23   et al., 2001).

24   In contrast to the relatively consistent findings for photosynthesis, O_3 effects on respiration

25   have been more variable. Stem respiration was unaffected by O_3 exposure (Matyssek et al.,

26   2002), suggesting that construction costs of new stems are not affected by O_3. However, the bole

27   represents a relatively large storage pool of carbohydrates in mature trees, and the timing of

28   phenological events among individual trees may help to confound the ability to statistically

29   detect differences in stem respiration across pollutant gradients (Grulke et al., 2001). Below-

30   ground respiration has been found to both increase and decrease in response to O_3, depending on

31   the approach and timing of CO_2 measures (Coleman et al., 1996; Andersen and Scagel, 1997;

1   Scagel and Andersen, 1997; King et al., 2001). The decreased soil respiration is thought to be

2   due to reduced root growth under O_3 exposure, but could also be partially explained by

3   decreased microbial respiration in response to O_3. Additional research is necessary to identify

4   the role of O_3 in affecting root vs. heterotrophic respiration, particularly over long time intervals.

5   Carbohydrate availability and use influence the degree to which plants respond to O_3

6   exposure. A model simulation of the effect of O_3 exposure on bole growth of *Pinus ponderosa*

7   showed a 15% reduction in mass (Weber and Grulke, 1995), largely influenced by differences in

8   carbohydrates allocated and partitioned in repair processes elsewhere in the tree. Foliar

9   respiration is thought to increase under elevated O_3 as maintenance costs (energy needs) of

10   leaves damaged by O_3 are higher than normal (Grulke and Balduman, 1999; Noormets et al.,

11   2001b), but differences in foliar respiration are subtle and difficult to detect statistically. Foliar

12   carbohydrate studies also suggest that more C is used under O_3 stress for repair processes (Topa

13   et al., 2001; Grulke et al., 2001) which would result in increased respiration. Ozone exposure

14   also reduced enzymatic activities of carbohydrate metabolism related to the breakdown of

15   sucrose (Einig et al., 1997). Changes in soil respiration in response to O_3, even though O_3 does

16   not penetrate into the soil, illustrates the tight coupling of plant C balance and soil biota and

17   illustrates the potential role O_3 plays in altering ecosystem C balances (Andersen, 2000).

18   Ozone can affect plant allometry through changes in energy use, potentially affecting net

19   primary production (NPP) at larger scales. The net effect of O_3 impacts on photosynthesis and

20   respiration for sensitive components of natural ecosystems is that height growth (Isebrands et al.,

21   2001; Oksanen, 2003b); and radial growth (Peterson et al., 1987, 1991; Isebrands et al., 2001;

22   Terrazas and Bernal-Salazar, 2002; Oksanen, 2003b) can be negatively affected by O_3. This has

23   been extrapolated to decreased NPP (Hogsett et al., 1997; Laurence et al., 2000).

24   Energy flow in plant communities can be altered by O_3 through changes in C allocation.

25   It is well known that elevated O_3 affects C allocation to roots (Coleman et al., 1995b; Andersen

26   et al., 1997; Grulke et al., 1998; Grulke and Balduman, 1999; Grulke et al., 2001) by decreasing

27   or inhibiting phloem loading of carbohydrates (Grantz and Farrar, 1999; Landolt et al., 1997), or

28   of carbohydrate metabolism (Einig et al., 1997). This leads to depressed root growth (Andersen

29   et al., 1991; Coleman et al., 1996; Grulke et al., 1998) and the potential for plant communities to

30   have an increased susceptibility to drought through altered root-shoot balance. Furthermore, it

31   can negatively affect below ground food webs (Scagel and Andersen, 1997; Phillips et al., 2002).

1   Another energetically costly response to O_3 exposure is that the production of defense

2   compounds, such as antioxidants, tend to increase under elevated O_3 conditions (Sheng et al.,

3   1997; Tausz et al., 1999b; 2002). Antioxidants help the plant scavenge free radicals before they

4   can cause damage to membranes or cell walls, but they demand C for production such that

5   growth can be adversely affected. In mature Jeffrey pine, stomatal uptake of O_3 elicited one

6   complex of antioxidant defenses in mesic microsites, while endogenously generated free radicals

7   in the chloroplast elicited a second complex of antioxidant defenses in xeric microsites (Grulke

8   et al., 2003b).

9

9.7.6.2 Material Flow

11   Plants as producers are responsible for using inorganic atmospheric C and reducing it into

12   organic forms used by consumers, thus driving nutrient processes in ecosystems. Ozone has the

13   potential to disrupt material flow through organic C cycling and changes in nutrient cycling,

14   particularly N and P cycling. Although there is indirect evidence that O_3 is disrupting C and

15   nutrient cycling at the ecosystem level, there is little direct evidence that O_3 alters nutrient

16   processing at ecosystem scales.

17   The greatest annual nutrient and C input to ecosystems is from foliar and root turnover.

18   Excision of plant parts and whole plant mortality are potentially much larger, but syncopated,

19   ecosystem inputs. Ozone exposure alters C cycling in the ecosystem by affecting the

20   within-plant C allocation and partitioning of dominant, O_3-sensitive plants, and through chemical

21   composition and rate of decomposition of sloughed plant parts (roots, branches, leaves)

22   (Figure 9-19).

23   In addition to O_3-induced changes in the quantity of C and nutrient inputs into ecosystems,

24   O_3 also can alter the nutrient quality of inputs. Ozone exposure alters nutrient levels in the

25   foliage (Boerner and Rebbeck, 1995; Lindroth et al., 2001; Fenn and Poth, 1998; Momen et al.,

26   2002) and affects the C:N ratio (Andersen et al., 2001; Lindroth et al., 2001; Grulke and Lee,

27   1997; Grulke et al., 2003b). Concentrations of compounds such as tannins, lignin and phenolics

28   (Kim et al., 1998; Findlay et al., 1996; Baumgarten et al., 2000; Saleem et al., 2001) are also

29   affected by O_3 exposure, which in turn alters decomposability (Fenn and Dunn, 1989) and litter

30   buildup in the ecosystem.

1   There are several possible pathways by which O_3 may affect litter quality and, hence, litter

2   decomposition, thus altering nutrient flow in ecosystems. These include altered C quality,

3   altered nutrient quality, and alteration of leaf surface organisms important in decomposition

4   pathways. For example, yellow poplar (*Liriodendron tulipifera*) and black cherry (*Prunus*

5   *serotina*) litter exposed to O_3 showed greater N loss during decomposition than charcoal filtered

6   controls, although mass loss did not vary among O_3 treatments (Boerner and Rebbeck, 1995).

7   Subsequent studies showed that although foliar N was not affected by O_3 exposure in yellow

8   poplar leaves, foliage decomposed more slowly (Scherzer et al., 1998). Other studies have also

9   shown a change in foliar N concentration in response to O_3 treatment, affecting the C/N ratio and

10 possible litter quality (Andersen et al., 2001).

11   In some cases, it appears that N remobilization from foliage into the plant is not complete

12 at the time of foliage abscission in O_3-exposed plants (Findlay and Jones, 1990; Stow et al.,

13 1992; Matyssek et al., 1993; Patterson and Rundel, 1995). Greater N content of senesced litter

14 could increase rates of decomposition. When O_3-exposed cottonwood (*Populus deltoides*) leaves

15 abscissed at the same time as control leaves, they decomposed at similar rates; however,

16 prematurely senesced foliage from O_3-exposed cottonwood decomposed more slowly than

17 controls despite their higher N content (Findlay and Jones, 1990; Findlay et al., 1991). Higher N

18 in senesced leaves appeared to be related to organic complexes formed by bound phenolics in

19 O_3-exposed leaves, that made the litter less palatable to decomposers, thereby slowing

20 decomposition rates (Jones et al., 1994; Findlay et al., 1996). Increased phenolics also have

21 been found in European silver birch (*Betula pendula*) exposed to O_3 (Saleem et al., 2001).

22   Carbon quality in leaf litter also changes in O_3-exposed foliage. Compositional changes in

23 leaf structural characteristics, such as lignin content, would be expected to alter rates of litter

24 decomposition (Fogel and Cromak, 1977; Meentemeyer, 1978; Kim et al., 1998). Blackberry

25 (*Rubus cuneifolus*) litter exposed to elevated O_3 had greater permanganate lignin than control

26 treatments, which was inversely related to mass-loss rates in decomposition studies (Kim et al.,

27 1998).

28   Ozone may affect early stages of decomposition by altering populations of leaf surface

29 organisms before or after senescence. Magan et al. (1995) found a shift in phyllosphere fungi on

30 Scots pine (*Pinus sylvestris*), Sitka spruce (*Picea sitchensis*), and Norway spruce (*Picea abies*)

31 exposed to O_3, but the potential effect of these changes on subsequent litter decomposition was

1   uncertain. The slowest decomposition rates of pre-exposed blackberry leaves were found when

2   senesced foliage was exposed to O_3 during decomposition, suggesting a possible direct effect of

3   O_3 on microorganisms in decomposing litter (Kim et al., 1998). Whether O_3 concentrations at

4   the soil surface influence initial stages of litter decomposition remains to be addressed.

5   Ozone exposure also reduces nutritional content of foliage because of the degradation of

6   chlorophyll. Reconstruction of chlorophyll may be limited by nutritionally poor soils or low soil

7   moisture, as well as alteration of root uptake by O_3 exposure and other stressors. Foliar exposure

8   to O_3 may also increase leaching of nutrients (Kerstiens and Lendzian, 1989). Ozone exposure

9   promotes early senescence of foliage (Miller and Elderman, 1977; Heath and Taylor, 1997), with

10   higher nutrient content than if excised later in the growing season (Poth and Fenn, 1998).

11   Since O_3 can slow decomposition through changes in leaf quality and quantity, leaf litter

12   can accumulate (Fenn and Dunn, 1989). The accumulation of soil organic matter from increased

13   leaf litter, even in the absence of acidic deposition, can lower soil pH (Binkley, 1992). Lower

14   soil pH can promote loss of nutrients, particularly cations, from the system, further reducing

15   nutrient availability to the plant. Complex organic compounds in decomposing litter may also

16   tie up nutrients, rendering them less available to plants.

17   Other factors such as nutrient deposition also affect the degree to which O_3 effects C and

18   nutrient flow through ecosystems. In high-pollution sites, the effect of N deposition is difficult

19   to separate from O_3 exposure, and reviews of the effect of acidic (and N) deposition on

20   ecosystem nutrient dynamics are important to consider (see Binkley, 1992; Fenn et al., 1998;

21   2003). For example, at moderately high pollution sites, foliar content of N is higher than that at

22   lower pollution site, but so are P, Mg, and Fe contents (Poth and Fenn, 1998). Although

23   significant changes in foliar tissue chemistry have occurred in response to long-term pollutant

24   deposition in the Carpathians (Mankovska et al., 2002; Fenn et al., 2002), much of this response

25   is correlated to heavy metal and N and S deposition. At this point, the contribution of O_3

26   exposure alone cannot be isolated without careful between-site comparisons of plant response

27   and understanding the depositional velocities of constituent atmospheric species. The significant

28   effects of plant response along known pollution gradients are important to consider because

29   heavy metal and N and S deposition has significantly declined over the last decade, while O_3

30   exposures remain high and declines in forest health have been sustained.

1   Models provide a means to track material flows through ecosystems. Two biogeochemical

2   models were parameterized to capture long-term effects of O_3 exposure, N deposition, and

3   climate on a *ponderosa pine*-dominated site in the eastern San Bernardino Mountains.

4   Simulated O_3 exposure resulted in faster production and turnover of foliage and a shift in carbon

5   from the canopy (15% reduction) to the forest floor (increase of 50 to 60%) (Arbaugh et al.,

6   1999). When O_3 exposure was combined with that of N deposition, litter mass exponentially

7   increased.

8   The direct effect of O_3 exposure on below-ground nutrient dynamics and ecosystem

9   material flow is poorly understood. Additional research will be necessary to understand spatial

10   and temporal dynamics of nutrient and C flow in ecosystems and to separate the effects of O_3

11   from those attributable to N deposition.

12

9.7.7 Hydrological and Geomorphological

14   At present, there are no publications on the effects of O_3 exposure that are carried through

15   at the ecosystem level to changes in mass water flow, channel morphology, riparian habitat

16   complexity, or sediment movement. It is possible that processes occurring at smaller scales are

17   affecting geomorphological processes in ecosystems; however, difficulty in scaling these

18   responses spatially and temporally have made it difficult to show experimentally. It is possible

19   that O_3 exposure affects water quality through changes in energy and material flows, as

20   discussed previously.

21

9.7.8 Natural Disturbance Regimes

23   There has been little research on how natural disturbances interact with O_3 to affect

24   performance of plants, communities, and ecosystems. The frequency, intensity, extent, and

25   duration of natural disturbances are variable and unpredictable. However, there have been

26   enough ecophysiological studies to suggest that O_3 could predispose plant communities to

27   certain natural stresses, e.g., drought, stress, or extreme low temperature stress during the winter.

28   While several studies have shown that drought stress reduces O_3 uptake through stomatal

29   closure, evidence also suggests that O_3 can alter plant water use and susceptibility to drought.

30   In controlled studies, Reich and Lassoie (1984) showed that relatively low O_3 concentrations

31   could diminish stomatal control and alter water use efficiency. Ash trees (*Fraxinus excelsior*)

1   exposed to elevated O_3 had greater water use early in the growing season, but less water use late

2   in the growing season when exposed to elevated O_3 (Wiltshire et al., 1994). Under moderate

3   drought stress, *Picea abies* trees grown under elevated O_3 consumed water faster and showed

4   higher stomatal conductances than controls (Karlsson et al., 1995). Pearson and Mansfield

5   (1993) showed that successive O_3 episodes disrupted stomatal function, making *Fagus sylvatica*

6   seedlings more susceptible to drought. Previous year O_3 exposure was shown to have a

7   carryover effect in the following growing season for *Fagus sylvatica* (Pearson and Mansfield,

8   1994).

9   Few studies showing the effects of O_3 on water relations of field-grown trees are found in

10   the literature. However, Grulke et al. (2003a) examined the effects of O_3 on canopy transpiration

11   of *Pinus jeffreyi* from mesic and xeric microsites and found that trees from mesic sites had 20%

12   more O_3 uptake than those in the xeric sites. The authors also concluded that the mesic trees had

13   greater O_3 injury as evidenced by lower needle retention, whereas trees in xeric microsites had

14   greater chlorotic mottle. Chlorotic mottle induced by stomatal uptake of O_3 is indistinguishable

15   from that of endogenously produced oxidants resulting from partially closed stomata, a reduction

16   of CO_2 inside the leaf, and production of strong oxidizers within the chloroplast when excited

17   electrons are passed to O_2 instead of CO_2 under high light levels.

18   Trees living near the limits of their freezing-tolerance range may be especially susceptible

19   to predisposition of freezing injury by O_3 (Sheppard et al., 1989). However, *Pinus halepensis*

20   exposed to elevated O_3 had enhanced winter hardiness (Wellburn and Wellburn, 1994). As with

21   the seasonal carryover of drought susceptibility, the influence of elevated O_3 on freezing

22   tolerance is carried over from summer to winter. Such effects have been demonstrated for *Picea*

23   *sitchensis* (Lucas et al., 1988) and for *Picea rubens* (Waite et al., 1994). Sorting out the role of

24   elevated O_3 in contributing to frost or low-temperature damage in forests remains difficult due to

25   the presence of other factors that may affect senescence.

26

9.7.9 Scaling to Ecosystem Levels

28   The vast majority of literature describing O_3 effects comes from short-duration herbaceous

29   plant or tree seedling studies under controlled conditions. Scaling results from these studies

30   requires extrapolation over both space and time in order to understand the full extent of changes

31   in ecosystems. In addition to spatial, temporal, and age-related complexities, ecosystems are

1 composed of organisms whose lifetimes range from hours to centuries (Laurence and Andersen,

2 2003). Forested ecosystems are affected by environmental conditions such as water and nutrient

3 availability, as well as by intra- and interspecies competition. Therefore, direct experimentation

4 to determine the response of forested ecosystems is not simply a matter of determining the effect

5 of O_3 on individual mature trees. In addition, even if an experiment can be conducted,

6 extrapolation of the results across landscapes and regions remains challenging. Nonetheless,

7 models provide a means to explore possible long-term changes and to identify important

8 research uncertainties.

9 Approaches to scaling fall roughly into two categories: (1) process-based modeling to

10 extrapolate physiological responses to O_3 based on seedling studies and (2) field assessments

11 using surveys and growth correlations, often in association with stand-level models to address

12 ecosystem complexity. Comparatively good information is available on process level effects of

13 O_3 in seedlings and, therefore, some models offer the opportunity to use this information to scale

14 O_3 effects at the stand and regional scales (Hogsett et al., 1997; Fuhrer et al., 1997; Chappelka

15 and Samuelson, 1998; Laurence et al., 2000).

16

17 **9.7.9.1 Scaling from Seedlings to Mature Trees**

18 A number of investigators have used simulation models based on physiological processes

19 to integrate available data and predict the effects of O_3 on mature trees. Such models predict tree

20 growth by simulating fundamental mechanisms, rather than through a statistical analysis of

21 empirical data. For instance, the process of photosynthesis is simulated based on environmental

22 conditions and physiological characteristics, and then the fixed C is allocated to plant growth

23 using principles of plant physiology. Models based on mechanisms should be applicable across

24 wide areas if the important functional relationships are represented accurately in the models and

25 if the environmental conditions are accurately identified. The ability of six models (TREGRO,

26 CARBON, ECOPHYS, PGSM, TREE-BGC, and W91) to simulate the effects of climatic

27 change and O_3 have been reviewed by Constable and Friend (2000). Of these models, only

28 PGSM and TREGRO explicitly simulated the effects of O_3 on foliar processes.

29 The TREGRO model was used to simulate C allocation and tissue growth in seedlings and

30 mature red oak trees based on the experimental data discussed above (Weinstein et al., 1998).

31 For seedlings at 2×-ambient O_3, only the total nonstructural carbohydrate (TNC) storage pool

1   was predicted to be affected. For mature trees, large decreases were predicted for TNC, leaves,

2   stem, branch, and both fine and coarse roots. Most predicted effects in mature trees were

3   consistent with observations in the field, but the simulations overestimated the effect of

4   2×-ambient O_3 on root TNC and growth. The authors suggested that this discrepancy may

5   have been due to trees reducing respiration in response to O_3 stress, a response not simulated in

6   the model.

7   For *Abies concolor*, TREGRO was parameterized and simulated growth of a mature tree

8   for 3 years to test for effects of O_3 exposure and drought stress (Retzlaff et al., 2000).

9   Reductions in O_3 exposure-mediated carbon assimilation were translated to losses in whole tree

10   biomass that probably would not be detectable in the field. However, TNC levels in branch

11   tissue were simulated to be lowered by over 50%, and branch growth was reduced in a

12   moderately polluted site relative to a clean site. Low O_3 exposure (sufficient to decrease C

13   assimilation by 2.5%) and drought stress (25% reduction in annual precipitation, which is

14   common on a decadal scale) acted synergistically to reduce C gain of whole tree biomass of

15   *A. concolor*. Simulated results of the tests were comparable to effects found in OTCs for

16   seedlings and pole-sized trees in clean and moderately polluted sites.

17   Models such as TREGRO are usually parameterized from many different sources of data,

18   including chamber experiments and plantations, from seedlings to mature trees, making it

19   difficult to validate that they reproduce changes that occur as trees develop from seedlings to

20   maturity. To address this issue, physiological and growth data were collected from a natural

21   stand of *P. ponderosa* and used to parameterize the TREGRO model (Grulke and Retzlaff,

22   2001). Representative trees of each of five tree age classes were selected based on population

23   means of morphological, physiological, and nearest neighbor attributes. Seedlings were

24   observed to differ significantly from pole-sized and older trees in most physiological traits. The

25   changes in biomass with tree age predicted from the model closely matched those of trees in the

26   natural stand.

27   The PGSM model was used to simulate *Pinus ponderosa* seedling growth responses to O_3

28   exposure and drought stress (Chen et al., 1994). Drought stress was predicted to reduce the

29   effect of O_3 on growth, as was observed in the experimental data. The TREGRO model was

30   used to simulate responses of Pacific Coast and interior varieties of *P. ponderosa* to five

31   simulated O_3 exposures between subambient and 3×-ambient (Constable and Taylor, 1997).

1   Simulated growth of var. ponderosa was reduced more than that in var. scopulorum with all O_3

2   exposures. Drought was protective of O_3 exposure. Similar results were also found in a

3   relatively moist ponderosa pine plantation (Panek and Goldstein, 2001), whereas drought was

4   synergistically deleterious with cumulative O_3 exposure in a natural stand (Grulke et al., 2002b).

5   For *Pinus taeda*, the Plant Growth Stress Model (PGSM) was calibrated with seedling data

6   and then used to simulate the growth of mature trees over a 55 year period in the Duke Forest,

7   NC, using estimates of historical O_3 concentrations (Chen et al., 1998). Simulated stem diameter

8   and tree height were comparable to observed values. In another simulation using TREGRO,

9   loblolly pine was more sensitive (greatest reduction in C gain) to a peak O_3 episode in July

10   (Constable and Retzlaff 1997), whereas mature tulip poplar *(Liriodendron tulipifera)* was more

11   sensitive to a peak O_3 episode in August.

12   For *Populus tremuloides*, the ECOPHYS model was used to simulate the relative above-

13   ground growth response of an O_3-sensitive clone (259) exposed to square-wave variation in O_3

14   concentration (Martin et al., 2001). The model adequately simulated several effects of O_3,

15   including a greater effect on stem diameter than on stem height, earlier leaf abscission, and

16   reduced stem and leaf dry matter production at the end of the growing season. For *Acer*

17   *saccharum*, the TREGRO model was use to predict effects of a 10-year O_3 exposure on root and

18   stem growth of a simulated 160-year-old tree (Retzlaff et al., 1996). Twice-ambient O_3 exposure

19   (for Ithaca, NY) was predicted to deplete the TNC pools and reduce fine root production.

20

21   **9.7.9.2 Surveys, Growth Correlations and Stand-Level Modeling**

22   Stand-level studies have included surveys of O_3 symptoms, correlations of radial growth

23   with O_3 and other environmental factors, and regional scale modeling. In addition, open air O_3

24   exposure systems, such as those being used on *Populus tremuloides*-mixed stands in northern

25   Wisconsin (Karnosky et al., 2003a) and on *Fagus sylvatica* and *Picea abies* in Germany (Nunn

26   et al., 2002) offer an opportunity to examine larger plot sizes, older trees, and trees growing

27   under realistic competition. Plots along natural O_3 gradients, as have been used very effectively

28   in southern California forest studies (Miller and McBride, 1999) and with *P. tremuloides* stands

29   in the Great Lakes region (Karnosky et al., 1999), offer additional insights into ecosystem level

30   responses. Undoubtedly, however, simulation modeling will have to become an integral

31   component of research in order to predict adequately ecosystem responses to O_3 (Laurence and

1   Andersen, 2003). Results of these approaches are discussed below, organized into three U.S.

2   regions: (1) northern states (including the upper Midwest and the Northeast), (2) southeastern

3   states, and (3) western states (primarily California). A fourth section contains selected

4   information from Europe.

5

6   ***Northern and Midwestern United States.*** In recent years, the USDA Forest Service has

7   conducted systematic O_3 biomonitoring surveys in most north-central and northeastern states

8   (Smith et al., 2003; Coulston et al., 2003). Plots are located on a systematic grid, and trained

9   field crews evaluate up to 30 plants of up to six species that have foliar injury symptoms

10   diagnostic of O_3 damage. For the United States as a whole, injury has been found more often in

11   eastern than in interior or west-coast states. As expected, O_3 injury is more common and more

12   severe in areas with higher O_3 concentrations. Of sampled *Prunus serotina* plots, ~12% were

13   estimated to be at high risk for injury based on a injury index derived from the survey data

14   (Coulston et al., 2003). *P. serotina* was estimated to be at risk for injury on the Allegheny

15   Plateau and the Allegheny Mountains (in Pennsylvania, West Virginia, and Maryland), as well as

16   in the coastal plain of Maryland and Virginia.

17   Ozone concentration, foliar injury, and physiological traits were measured on *P. serotina*

18   trees of different sizes in Pennsylvania (Fredericksen et al., 1995). The proportion of foliage

19   injured was 46% for seedlings, 15% for saplings, and 20% for canopy trees. Cumulative O_3 flux

20   was the most useful O_3 metric for predicting injury. Injury was negatively correlated ($r^2 = 0.82$)

21   with net photosynthetic rates, but was not related to stomatal conductance. *P. serotina* is

22   discussed further below, because detailed surveys have been conducted in the Shenandoah and

23   Great Smoky Mountains National Parks in the southeastern United States.

24   Over the past several decades, some surveys of *Pinus strobus* have reported significant

25   associations between foliar injury and reduced growth (Anderson et al., 1988; Benoit et al.,

26   1982). However, a review of 93 surveys conducted from 1900 through the late 1980s concluded

27   that methodological problems were pervasive, including such issues as proximity to roads, lack

28   of peer review, lack of random sampling, small sample sizes, and lack of quantitative methods to

29   estimate severity (Bennett et al., 1994). Because of these problems, along with evidence of

30   adequate growth rates for *P. strobus* regionally and contradictory evidence from numerous

31   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 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 *Prunus serotina*, *Populus tremuloides*, *Fraxinus*

americana, *Pinus banksiana*, big-leaf aster, and spreading dogbane. Species showing foliar

injury at 1.5 × ambient O_3 concentrations included *Betula populifolia*, small sundrops, and

bunchberry. Species remaining uninjured at 2 × ambient O_3 concentrations included *Betula*

papyrifera, *Pinus strobus*, *Pinus rigida*, *Picea rubens*, *Thuja occidentalis*, *Quercus robur*,

Canada bluejoint grass, wild radish, and Canada mayflower. Because of their O_3 sensitivity and

diagnostic symptoms, big-leaf aster, spreading dogbane, *Populus tremuloides*, *Fraxinus*

americana, and *Prunus serotina* 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

1   drought stress was absent. Little variation was predicted among years, because high O_3 often

2   coincided with hot, dry weather conditions that reduced predicted stomatal conductance and

3   O_3 uptake.

4   In order to estimate the impact of O_3 on forests, effects must be evaluated not only on

5   individuals, but also on mixtures of species and the composition of forest stands. The PnET

6   model described above evaluated the effects of O_3 on broad forest types (an evergreen/deciduous

7   mix), but did not address specific forest species composition. In order to address competition

8   among species, the TREGRO model was linked to the ZELIG forest stand growth model and a

9   geographic information system was created to predict the effects of O_3 across the north-central

10   and northeastern United States (Laurence et al., 2000). ZELIG is a gap-succession model used

11   to simulate succession in mixed stands typical of eastern and northern forests. Ambient O_3

12   generally caused a reduction of 2 to 4% in the growth of *Quercus robur* across the region during

13   the 100-year simulation. The response followed the pattern of O_3 exposure, with little effect in

14   the northwest part of the region, but with greater effect in southern locations. The O_3 response of

15   *Acer saccharum* to O_3 varied widely, but the overall growth response was always positive,

16   indicating that the evergreen/deciduous mix was able to take advantage of the decrease in the

17   growth of *Q. robur* and other species caused by O_3. In the northernmost part of the region,

18   *A. saccharum* growth increased by up to 3%, but in the southern part of the region, its growth

19   increased by up to 12%. The authors ascribed this enhanced growth to a combination of warmer

20   temperatures and reduction in the growth of *Prunus serotina*, a minor component of the

21   simulated stand that was very sensitive to O_3.

22

23   ***Southeast United States.*** In a survey of the Great Smoky Mountains National Park, foliar

24   injury attributed to O_3 was found on 47% of the more than 1,600 plants examined (Chappelka

25   et al., 1997). In subsequent surveys of injury in the park, injury was found on mature trees of the

26   following species: *Sassafras albidum*, *Prunus serotina*, and *Liriodendron tulipfera* (Chappelka

27   et al., 1999a,b). In a similar study in Shenandoah National Park, injury was found on *Fraxinus*

28   *americana*, *P. serotina*, and *L. tulipfera* (Hildebrand et al., 1996).

29   For *Prunus serotina* seedlings grown in soil in OTCs and exposed to relatively low

30   ambient levels of O_3 in Pennsylvania, there was no correspondence between visible foliar stipple,

31   leaf gas exchange, and seedling growth between two families previously shown to differ in O_3

1   symptoms (Kouterick et al., 2000). However, significant exposure-response relationships were

2   found for foliar injury in the Great Smoky Mountains and Shenandoah National Parks. In each

3   park, foliar injury was evaluated on mature *P. serotina* trees on three plots at different elevations

4   near O_3 monitors during 1991 to 1993 (Hildebrand et al., 1996; Chappelka et al., 1999a,b).

5   In 1991, incidence was 60% and 45% for the two parks and 33% in both parks during 1992 and

6   1993. Symptoms were greater at the highest elevations where O_3 concentrations were highest.

7   In another study, radial growth rates were measured in 44 *P. serotina* trees ranging in age from

8   19 to 56 years old with and without O_3 symptoms, at three sites in the Great Smoky Mountains

9   National Park. Trees with O_3 symptoms were compared to similar-sized trees with few

10   symptoms. There was no evidence that trees with O_3 symptoms had lower growth rates (p = 0.6)

11   (Somers et al., 1998).

12   In the Great Smoky Mountains National Park, radial growth rates were measured for

13   44 *L. tulipfera* trees ranging in age from 30 to 58 years old, with and without O_3 symptoms, at

14   three sites at different elevations (Somers et al., 1998). Trees with O_3 symptoms averaged 30%

15   lower growth rates over ten years (p = 0.0005). Seedlings of *Liriodendron tulipfera* were

16   exposed for two seasons to 2×-ambient O_3 exposures in OTCs in Delaware, OH (seasonal

17   SUM00 exposures of 107 and 197 ppmh) (Rebbeck, 1996). Foliar O_3 symptoms were observed,

18   but growth was not reduced.

19   In order to evaluate the influence of interspecies competition on O_3 effects, the linked

20   TREGRO and ZELIG modeling system was used to predict the effects of O_3 over 100 years on

21   the basal area of species in a *Liriodendron tulipfera*-dominated forest in the Great Smoky

22   Mountains National Park (Weinstein et al., 2001). Ambient O_3 was predicted to reduce the basal

23   area of *L. tulipfera* by 10%, whereas a 1.5×-ambient exposure was predicted to cause a 30%

24   reduction. Basal area of *Acer rubrum* and *Prunus serotina* was predicted to increase for some

25   years, but then decrease by up to 30%, with few changes in the total basal area of all species by

26   the end of the simulation.

27   In order to evaluate the influence of interspecies competition on O_3 effects, the linked

28   TREGRO and ZELIG modeling system was used to predict the effects of O_3 on the basal area of

29   *Pinus taeda* and *Liriodendron tulipfera* growth throughout their ranges (Laurence et al., 2003).

30   The models were parameterized using biological and meteorological data from three sites in the

31   southeastern United States (in Alabama, Louisiana, and North Carolina). Forest stand response

1   to five O_3-exposure regimes with annual SUM06 values ranging from 0 to 100 ppmh per year

2   was simulated for 100 years. The simulated basal area of the two species was generated within

3   the context of four other tree species common in southeastern forests. Basal area of *P. taeda* was

4   highly responsive to precipitation and O_3 exposure, with the greatest increases under high-

5   precipitation, lowO_3-exposure scenarios and the greatest decreases under low-precipitation, high

6   O_3-exposure scenarios. The basal area of *L. tulipifera* did not significantly differ (+10%) from

7   simulations using a "base case" (ambient O_3, average precipitation).

8   Systematic biomonitoring surveys found that approximately 24% of sampled sweetgum

9   and 15% of sampled *Pinus taeda* plots were estimated to be at high risk for foliar injury on the

10   coastal plain of Maryland and Virginia (Smith et al., 2003; Coulston et al., 2003). In a study in

11   Tennessee, the effect of ambient (uncontrolled) O_3 on 28 mature canopy-dominant 50 to

12   90-year-old *P. taeda* trees in five stands was measured over a 5-year period (McLaughlin and

13   Downing, 1995, 1996). Of many O_3 metrics, a 3-day average of hourly O_3 values $\geq$ 40 ppb

14   (AOT40) was found to best explain short-term variation in stem expansion as measured with

15   dendrometer bands. Interactions between O_3, temperature, and drought stress (as indicated by

16   the weekly moisture stress index) accounted for 63% of the short-term variation in stem growth

17   rates. Because there are interactions among O_3, drought stress, and temperature that may differ

18   with the averaging time (days to years), this type of study cannot provide conclusive proof of

19   cause and effect (Reams et al., 1995). However, the results do suggest that the effects of O_3

20   measured on loblolly seedlings may also be occurring in mature trees in both wet and dry sites.

21   The magnitude of effects of O_3 on growth, including interactions with other variables in this

22   study, ranged from 0 to 15% over 5 years, with an average of 5.5%.

23

24   ***Western United States.*** The USDA Forest Service conducted O_3 biomonitoring surveys in

25   Washington, Oregon, and California during one year (1998), and in the Sierra Nevada and

26   Sequoia National Forest every other year for several decades (Campbell et al., 2000). Overall,

27   only one plot showed any symptoms of O_3 injury outside of the Sierra Nevada and Sequoia

28   National Forests. In the Sierra Nevada National Forest, between 30 and 40% of trees showed

29   injury from 1989 through 1997. In the Sequoia National Forest, between 40 and 50% of the

30   trees surveyed showed injury from 1990 through 1998.

1   For *Pinus ponderosa* along a well-studied gradient of O_3 exposure in the San Bernardino

2   mountains, chlorotic mottle was highest on foliage at the most polluted site, as has been found

3   previously (Grulke and Balduman, 1999). Based on whole-tree harvests, root biomass was

4   lowest at the most polluted sites, confirming previous studies with seedlings under controlled

5   conditions, as discussed above. Ozone responses in highly polluted environments such as

6   Southern California may not be predicted adequately by extrapolating effects from single-factor

7   experiments. Instead, combined approaches utilizing field experiments and modeling efforts

8   may be required to properly account for a combination of stressors including O_3, N deposition,

9   and drought. Furthermore, the available studies underscore the lack of correlation between O_3

10   symptoms and mature tree effects. If a field survey fails to find a correlation between mature

11   tree growth and O_3, this result may be due to the dominant effect of another factor such as N

12   deposition and may not be evidence that O_3 does not reduce the growth of mature trees.

13   The TREGRO model was used to evaluate how projected future temperature and CO_2

14   concentrations might affect the response of individual ponderosa pine to O_3 at seven sites in

15   California, Oregon, and Washington (Tingey et al., 2001). As expected, growth decreased with

16   increasing O_3 exposure. Differences in O_3 response among sites appeared to be due primarily to

17   differences in precipitation.

18   Often air quality standards do not translate directly into measureable improvements in tree

19   growth or productivity. To evaluate whether past improvements in air quality have improved

20   ponderosa pine growth, TREGRO was used to simulate growth at sites in the San Bernardino

21   Mountains in California (Tingey et al., 2004). Ozone and meteorogical data from the past

22   37 years was used to run the simulations. Despite variation in precipitation and temperature,

23   O_3 was found to reduce simulated tree growth. The authors were able to simulate growth

24   improvements as air quality improved during the 1980s and 1990s, suggesting that

25   improvements in emission control strategies benefited ponderosa pine. The model simulations

26   were qualitatively consistent with improvements in canopy condition that were observed at sites

27   where O_3 reductions were the greatest.

28

29   ***Studies in Europe.*** In a 4-year study of *Fagus sylvatica* in Switzerland at 57 forest sites

30   ranging in age from 65 to 173 years, stem increment was found to decrease with increasing

31   maximum O_3 exposure (Braun et al., 1999). In this study, O_3 concentration was estimated by

1   interpolation among monitoring stations, and other site conditions such as soil water status and

2   temperature were interpolated from weather stations. Other factors such as N deposition, tree

3   diameter, and canopy dominance were also found to be significantly associated with stem

4   increment. The maximum annual O_3 dose (expressed as AOT40) was found to be more strongly

5   associated with decreased stem increment than was the average O_3 dose over the 4 years.

6   A growth reduction of 22.5% (confidence interval 14.3 to 28.6%) was associated with each

7   10 ppmh increment of O_3 (expressed as AOT40). This decrease was steeper than the 6.1%

8   growth reduction summarized previously from several OTC studies with *F. sylvatica* seedlings

9   (Fuhrer et al., 1997). However, the authors suggest that this difference may be explained largely

10   by the 4 years of exposure in their forest survey study as compared to the one-year exposures for

11   seedlings. As with any forest survey, these results must be interpreted with caution because O_3

12   exposure was correlated with other variables, such as tree age and the deposition of NO_2

13   and SO_2.

14

9.7.10   Summary of Ecological Effects of Ozone Exposure on Natural Ecosystems

17   In this chapter, an effort has been made to discuss the adverse effects of O_3 on natural

18   ecosystems within the context of the SAB framework for assessing and reporting ecological

19   conditions (Young and Sanzone, 2002). Using this framework, there is evidence that

20   tropospheric O_3 is an important stressor of natural ecosystems, with well-documented impacts on

21   the biotic condition, ecological processes, and chemical/physical nature of natural ecosystems

22   (Figure 9-20). In turn, the effects of O_3 on individual plants and processes are scaled up through

23   the ecosystem affecting processes such as energy and material flow, intra and interspecies

24   competition, and NPP. Thus, effects on individual keystone species and their associated

25   microflora and fauna may cascade through the ecosystem to the landscape level. This suggests

26   that by affecting water balance, cold hardiness, tolerance to wind, and by predisposing plants to

27   insect and disease pests, O_3 may even influence the occurrence and impact of natural

28   disturbance. Despite the probable occurrence of such effects, however, there are essentially no

29   instances where highly integrated ecosystem-level studies have conclusively shown that O_3 is

30   indeed altering ecosystem structure and/or function.

31

Stressors (top left box)
- Hydrolic Alteration
- Habitat Conversion
- Habitat Fragmentation
- Climate Change
- Invasive Non-native Species
- Turbidity/Sedimentation
- Pesticides
- Disease/Pest Outbreaks
- Nutrient Pulses
- Metals
- Dissolved Oxygen Depletion
- Ozone (Tropospheric)
- Nitrogen Oxides
- Nitrates

Stressors (top middle box)
- Hydrolic Alteration
- Habitat Conversion
- Habitat Fragmentation
- Climate Change
- Over-Harvesting Vegitation
- Large-Scale Invasive Species Introduction
- Large-Scale Disease/Pest Outbreaks

Stressors (right upper box)
- Hydrolic Alteration
- Habitat Conversion
- Climate Change
- Over-Harvesting Vegitation
- Disease/Pest Outbreaks
- Altered Fire Regime
- Altered Flood Regime

Hexagon labels: Landscape Condition, Biotic Condition, Natural Disturbance, Chemical / Physical, Hydrology / Geomorphology, Ecological Process

Stressors (lower left box)
- Hydrolic Alteration
- Habitat Conversion
- Climate Change
- Turbidity/Sedimentation
- Pesticides
- Nutrient Pulses
- Metals
- Dissolved Oxygen Depletion
- Ozone (Tropospheric)
- Nitrogen Oxides
- Nitrates
- Sulfates
- Salinity
- Acidic Deoosition

Stressors (bottom middle box)
- Hydrolic Alteration
- Habitat Conversion
- Climate Change
- Pesticides
- Disease/Pest Outbreaks
- Nutrient Pulses
- Dissolved Oxygen Depletion
- Nitrogen Oxides
- Nitrates
- Sulfates

Stressors (lower right box)
- Hydrolic Alteration
- Habitat Conversion
- Habitat Fragmentation
- Climate Change
- Turbidity/Sedimentation

Figure 9-20. Common anthropogenic stressors and the essential ecological attributes they affect.

Source: Modified from Young and Sanzone (2002).

1   Systematic injury surveys demonstrate that foliar injury occurs on sensitive species in

2 many regions of the United States. However, the frequent lack of correspondence between foliar

3 symptoms and growth effects means that other methods must be used to estimate the regional

4 effects of O_3 on tree growth rates. Investigations of the radial growth of mature trees in

5 combination with data from many controlled studies with seedlings, as well as a few studies with

1   mature trees suggest that ambient O_3 is reducing the growth of mature trees in some locations.

2   Studies using models based on tree physiology and forest stand dynamics suggest that modest

3   effects of O_3 on growth may accumulate over time and may interact with other stresses. For

4   mixed-species stands, such models predict that overall stand growth rate is generally not likely to

5   be affected. However, competitive interactions among species may change as a result of growth

6   reductions of sensitive species. These results suggest that O_3 exposure over decades may be

7   altering the species composition of forests in some regions.

8

9   **RESEARCH NEEDS**

10   The knowledge base for examining the range of ecological effects of O_3 on natural

11   ecosystems is growing, but significant uncertainties remain regarding O_3 effects at the ecosystem

12   level. For example, there is a need for information on the following ecosystem-level responses:

13   - *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).

14   - *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 (Pitelka, 1988; Winner
     et al., 1991; Davison and Barnes, 1998). 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.

15   - *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.

16   - *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.

1   • *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 (Skarby et al., 1998).

2   • *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).

3   • *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).

4   • *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.

5   • *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).

6

7

8   **9.8 ECONOMIC EVALUATION OF OZONE EFFECTS ON**
9   **AGRICULTURE, FORESTRY AND NATURAL ECOSYSTEMS**

10   **9.8.1 Introduction**

11   The adverse consequences of ambient of air pollutant exposures on vegetation, ecosystems

12   and components of the material environment have been documented since the beginning of the

13   industrial revolution. Attempts to quantify the monetary damage and injury resulting from

1 tropospheric O_3 exposures to managed agriculture, forests, and natural ecosystems date back at
2 least to the 1950s.

3   Both methodological and data problems plagued early efforts to assess the monetary
4 damages of air pollution to crops and natural vegetation. Adams and Crocker (1989) discussed
5 the methodological issues, e.g., a lack of reliable data on effects from air pollutants on crop
6 yields or the failure to develop and apply appropriate economic models. Some of these problems
7 were remedied by the EPA's National Crop Loss Assessment Network (NCLAN) in the 1980s.
8 The EPAs NCLAN facilitated the performance of economic assessments by providing O_3-crop
9 yield data with which to estimate O_3-crop yield response functions (see Heagle [1988] for a
10 review of NCLAN procedures and findings). NCLAN also funded a series of economic
11 assessments that, along with subsequent economic assessments, documented substantial
12 economic damages to agriculture. (See Spash [1997] for a detailed review of economic
13 assessments, many of which used NCLAN data.)

14   Since the completion of the NCLAN program in the late 1980s, the number of economic
15 assessments of air pollution studies focusing on terrestrial ecosystems in general, and agriculture
16 in particular, has declined. For example, for the period of 1980 to 1990, 33 economic studies of
17 O_3 and other air pollutant effects on U.S. crops were published in peer-reviewed journal outlets
18 (Spash, 1997). However, in preparing this section of the current criteria document, only four
19 peer-reviewed economic assessments were found for the decade of 1991 to 2000 that addressed
20 vegetation in the United States. In addition, one peer-reviewed article (Kuik et al., 2000) was
21 found dealing with agriculture in the Netherlands. Recent interest in global climate change and
22 the potential effects of global warming on O_3 and other photochemical oxidants, has renewed
23 interest in the effects of air pollution on both managed and unmanaged terrestrial ecosystems
24 (Adams et al., 1998). In addition, concern is growing for regarding the effects of air pollutants
25 on natural ecosystems and on the services they provide (Daily, 1997). Unfortunately, this
26 interest has not yet translated into additional peer-reviewed publications addressing O_3 or other
27 air pollutants effects on ecosystems.

28   This section of the current criteria document first discusses the availability of economic
29 information and its usefulness in forming environmental policy. Next, economic assessments of
30 air pollution effects and findings from the 1996 AQCD (U.S. Environmental Protection Agency,
31 1996) are discussed, followed by a synthesis of the limited literature available since the 1996

1 AQCD with respect to O_3 effects on agriculture, forestry, and ecosystems. Finally, limitations

2 and continuing uncertainties are reviewed. The most fundamental of these is the lack of

3 measurements of the economic effects of air pollution on natural ecosystems. Other issues

4 include the variability of performance in both managed and natural ecosystems under increased

5 climatic and air pollution variability as well as the challenges related to spatial and temporal

6 scales used in performing economic assessments. To date, this set of effects has been sparsely

7 addressed.

8

9.8.2   The Measurement of Economic Information

10 Economic science is an exercise in deductive logic in which testable hypotheses about the

11 behavior of economic agents (i.e., farmers, consumers, resource owners) and markets are

12 deduced from a body of theory. That body of theory is based on a series of premises proposed

13 by economists and philosophers dating back over two centuries to Adam Smith. These premises

14 gradually evolved into a theoretical foundation primarily dealing with microeconomics,

15 culminating in structural relationships that define the operation of markets. This foundation was

16 first laid out in a comprehensive and rigorous fashion by Alfred Marshall in 1920. Paul

17 Samuelson (1948) formalized these theoretical relationships, resulting in what is sometimes

18 referred to as modern, or neoclassical, economics.

19 The insights gained from the theoretical foundation of economics helped shape the nature

20 of applied economic, or policy-relevant research. An example of such applied research is when

21 economists seek to measure the economic consequences of air pollution on agriculture. Such an

22 application is described in Adams and Horst (2003), who provided a graphical representation of

23 the measurement of the effects of air pollution on the well-being of producers and consumers.

24 Economic theory is applied to real-world problems when the methods of economics and the need

25 for data from other disciplines come into play. When measuring the economic effects of an

26 environmental change, economists need an economic model or method that is theoretically

27 consistent, i.e, defensible, as well as data with which to estimate both economic and

28 environmental science relationships for use in the model. Among accepted economic assessment

29 methods, the actual choice is frequently determined by the nature of the problem to be addressed.

30 It should be noted that the choice of assessment method can affect the type of economic

31 information that is obtained. Even with a given assessment method, results are sensitive to

1   specific data treatment or assumptions (Adams, 1999). For example, some methods only

2   measure effects on a particular group e.g., farmers. Other methods may measure effects across

3   several groups. Thus, one should not expect the magnitudes of damage or benefits to be

4   identical across economic assessments. One should, however, expect that the direction of the

5   effects will be similar.

6   Once it is established that an assessment meets basic economic criteria, e.g., including

7   human behavioral responses, the selection of the specific economic assessment method is often a

8   relatively minor issue in terms of estimating benefits of air pollution control (or disadvantages of

9   increases in air pollution). Although results differ across approaches, the differences are largely

10   attributable to specific features of the assessment (e.g., whether the natural science data include a

11   particular effect or relationship, whether effects on consumers are measured, and so forth). The

12   nature and quality of the air quality forecasts used in the assessments can greatly influence the

13   sensitivity of the assessments (Adams and Crocker, 1989). This is particularly noticeable when

14   dealing with forecasts of seasonal air pollution changes (Adams et al., 1988). From the

15   standpoint of providing policy guidance, the differences in economic estimates attributable to the

16   assessment methods are often swamped by uncertainty in the natural and physical science

17   forecasts. This has also been noted in recent economic assessments of climate change (Adams

18   et al., 1998). In many settings, the quality of economic assessments of air pollution is likely to

19   be improved more by refining the physical and natural science data used in the assessments than

20   by intensive efforts to fine-tune the assessment techniques (Adams, 1999).

21

22   ### 9.8.3 Understanding of Air Pollutants Effects on the Economic Valuation of
23   Agriculture and Other Vegetation in the 1996 Criteria Document

24   Evidence from the plant science literature cited in the 1996 O_3 AQCD (U.S. Environmental

25   Protection Agency, 1996) is unambiguous with respect to the adverse effects of tropospheric O_3

26   on some types of vegetation. For example, the 1996 AQCD noted that findings from the EPA

27   multiyear NCLAN program in the 1980s provided rigorous corroboration of at least two decades

28   of previous research and a century of anecdotal observations that showed that O_3 at ambient

29   levels caused physical damage to plants in general and to important crop species in particular.

30   Specifically, NCLAN established that ambient O_3 levels resulted in statistically significant

31   reductions in yields for some crop species (Heagle et al., 1988). The 1996 AQCD also assessed

1   the results of studies regarding O_3 effects on crops, forests, and natural vegetation in more detail.

2   More recent reviews, such as the comprehensive survey of the economic literature on

3   agricultural effects by Spash (1997) corroborate the synthesis of results reported in the 1996

4   AQCD.

5   The number and quality of assessments of the economic consequences of O_3 exposures on

6   vegetation reported in the 1996 AQCD are primarily a function of the state of evidence obtained

7   from scientific studies in each vegetation category. For example, the plant science evidence

8   reviewed in the 1996 AQCD concerning effects of O_3 exposures on agricultural crops was

9   reported to be more valid than for individual tree species or plant communities (ecosystems).

10   As a result, most economic assessments discussed in the 1996 AQCD focused on the data

11   obtained from studies of agricultural crops. The economic literature dealing with O_3 effects on

12   forest productivity in the 1996 AQCD is sparse. The few economic assessments of tree or forest

13   effects reported in the 1996 AQCD were confined to evaluations of assumed or hypothetical

14   changes in output, such as board feet of lumber (e.g., Haynes and Adams [1992]). As noted in

15   the 1996 AQCD, O_3 effects on ecosystems and their services had not been measured in any

16   systematic fashion and no peer-reviewed economic assessments were yet reported.

17   This section first briefly reviews economic assessments drawn from the review in the 1996

18   criteria document. This review is the benchmark against which recent articles are then discussed

19   in the subsequent section. As was the case in 1996, the discussion of economic valuation of

20   ecosystem effects is generally limited to conceptual and methodological issues, given the

21   continued lack of empirical analyses in this category.

22

23   **9.8.3.1 Agriculture**

24   In view of the importance of U.S. agriculture for both domestic and world consumption of

25   food and fiber, reductions in U.S. crop yields could adversely affect human welfare. The

26   plausibility of this premise has resulted in numerous attempts to assess, in monetary terms, the

27   losses from ambient O_3 exposures or the benefits of O_3 control, to agriculture. Twenty-three

28   assessments of the economic effects of O_3 exposures on agriculture were reviewed in the 1996

29   AQCD, highlighting key issues in the validity of these assessments (U.S. Environmental

30   Protection Agency, 1996). First, the evidence should reflect how crop yields will respond under

31   actual field conditions to O_3 exposures. Second, the air quality data used to frame current or

1   hypothetical effects of O_3 on crops should represent actual exposures sustained by crops at

2   individual sites or production areas. Finally, the assessment methodology into which such data

3   are entered should (1) capture the economic behavior of producers and consumers as they

4   adjust to changes in crop yields and prices that may accompany changes in O_3 air quality;

5   (2) accurately reflect institutional considerations, such as regulatory programs and income

6   support policies (e.g., provisions of federal Farm Bill legislation), that may result in market

7   distortions; and (3) use measures of well-being that are consistent with economic principles.

8   Assessments of O_3 damage to agricultural crops reported in the 1996 AQCD employed

9   procedures for calculating economic losses that met the conditions described above. More

10 specifically, the assessments provided 23 quantitative estimates of the economic consequences

11 of exposures to O_3 and other air pollutants to agriculture that reflect producer-consumer

12 decision-making processes, associated market adjustments, and some measure of distributional

13 consequences between affected parties. Many of the economic assessments reviewed in

14 previous O_3 documents also focused on O_3 effects in specific regions, primarily California and

15 the Corn Belt (e.g., Garcia et al., 1986). This regional emphasis in the earlier literature may be

16 attributed to the relative abundance of data on crop response and air quality for selected U.S.

17 regions, as well as the importance of some agricultural regions (such as California) for the U.S.

18 agricultural economy.

19   Two U.S. national studies described in previous O_3 criteria documents that are worthy of

20 additional comment are Kopp et al. (1985) and Adams et al. (1986). These were judged to be

21 "adequate" in terms of the three critical areas of data inputs in the 1996 AQCD. Together, it was

22 argued, they provide a reasonably comprehensive estimate of the economic consequences of

23 changes in ambient air O_3 levels on agriculture. Because of their central role in the 1996 criteria

24 document, these two studies are reviewed briefly below.

25   The Kopp et al. (1985) and Adams et al. (1986) studies indicated that ambient levels of O_3

26 were imposing substantial economic costs of ~$3.4 billion (in 2000 U.S. dollars) on agriculture.

27 Both were judged to suffer from several sources of uncertainty, but the document concluded that

28 these possible improvements in future assessments were not likely to greatly alter the range of

29 agricultural benefit estimates arising from O_3 reductions for several reasons. First, the studies

30 covered about 75 to 80 % of U.S. agricultural crops (by value). For inclusion of the other 20%

31 to significantly change the estimates would require that their sensitivities to O_3 be much greater

1   than for the crops that have been included to date. Second, model-sensitivity analyses reported

2   in past studies indicate that changes in plant exposure response relationships must be substantial

3   to translate into major changes in economic benefits estimates. For example, it was assumed

4   unlikely that use of different exposure measures, or inclusion of interaction effects, would

5   greatly alter the magnitude of the economic estimates. Third, it was believed that countervailing

6   effects would mitigate against large swings in the estimates, e.g., longer O_3-exposure periods

7   may predict greater yield losses, but O_3-water stress interactions tend to reduce loss the

8   yield estimates.

9   Other national assessments reported in the 1996 AQCD provided general corroboration of

10   the results of Kopp et al. (1985) and Adams et al. (1986). An evaluation of these studies in

11   terms of the adequacy of information from plant exposure studies, and aerometric and economic

12   data was presented in the 1996 AQCD, along with estimates of benefits or damages associated

13   with changes in O_3. Most of the studies built on either Kopp et al. (1985) or Adams et al. (1986).

14   A relevant question was whether subsequent studies provided any "surprises" in terms of

15   magnitude of economic effects.

16   Common themes or findings from these and earlier O_3 and other air pollution studies were

17   summarized in two synthesis papers, Adams and Crocker (1989) and Segerson (1991). The

18   major conclusion is that the agricultural effects of tropospheric O_3 at ambient levels impose

19   economic costs to society or, conversely, that reductions in ambient O_3 should result in societal

20   benefits.

21   Several studies contained in the 1996 AQCD still are of interest. For example, one finding

22   pertains to the relationship between federal farm programs and air pollution regulations

23   (McGartland, 1987). In each case, the inclusion of farm programs in the economic models

24   resulted in modest reductions in the economic benefits of O_3 control due to increased farm

25   program costs. As Segerson (1987) noted, however, it is not clear that these increased costs

26   should be charged against the potential benefits of an O_3-regulatory standard, but rather,

27   considered as an additional cost associated with the inefficiencies of the farm program. It should

28   also be noted that the nature of federal farm programs was changed dramatically by Congress in

29   1996 in an attempt to reduce the federal government's role in agriculture. Although more recent

30   federal legislation, such as the 2002 Farm Bill (U.S. Congress, 2002), appears to be restoring the

31   federal government's role in the farming sector, this issue currently is not as important as

1　　suggested by earlier studies, due to the declining reliance on deficiency payments to farmers,

2　　which tend to distort resource allocation.

3　　　　Another national study (Adams et al., 1988) analyzed economic benefits under a regulatory

4　　alternative involving a seasonal (crop growing season) O_3-exposure index measured as a 12-h

5　　mean, instead of hourly levels or percent changes from ambient as reported in earlier studies.

6　　Specifically, a seasonal average of 50 ppb O_3 (measured as a 12-h seasonal mean) with a

7　　95% compliance level, is reported in Adams et al. (1988). The result (a $2.9 billion benefit in

8　　2000 dollars) is similar to the assumed 25% reduction across all regions reported by Adams et al.

9　　(1986). At least one study also combined environmental stressors (e.g., O_3, UV-B radiation) in

10　　performing economic assessments. Adams and Rowe (1990), using the same model as Adams

11　　et al. (1986), reported that a 15% depletion of stratospheric O_3 (resulting in a 13% increase in

12　　tropospheric O_3) would cause an economic loss of ~$1.4 billion in 2000 dollars attributed to the

13　　tropospheric O_3 increase. Reducing VOCs/NO_x motor vehicle emissions by 10% would result in

14　　a benefit of ~$0.3 billion, while a complete elimination of motor vehicle emissions would yield a

15　　benefit of ~$3.4 billion (1990 dollars). The range of these numbers is consistent with values

16　　reported in Adams et al. (1986), Kopp et al. (1985), and other national-level analyses, i.e.,

17　　estimates of from $1.0 to 2.0 billion for reductions in ambient O_3 of 25 to 40%.

18

19　**9.8.3.2　Forests (Tree Species) and Natural Ecosystems**

20　　　　The long-term nature of air pollution effects on perennial species such as trees creates

21　　challenges to plant scientists in attempts to sort out the effects of specific individual stressors

22　　such as O_3 from among the many other potential causal factors (Skelly, 1988). It also creates

23　　problems in terms of measuring the impacts on direct economic value, such as reductions in

24　　board-feet of lumber produced per unit of time.

25　　　　Most of the literature in the 1996 AQCD dealing with forest species reported the effects of

26　　O_3 exposures in terms of foliar injury (Davis and Skelly, 1992; Freer-Smith and Taylor, 1992;

27　　Simini et al., 1992; Taylor, Jr. and Hanson, 1992). This emphasis on foliar effects in the forest

28　　effects literature (rather than marketable yield) is similar to the state of science for agricultural

29　　crops prior to 1980. More recent studies address the effects of air pollutants on forest tree

30　　species diversity (Bringmark and Bringmark, 1995; Vacek et al., 1999; Weiner et al., 1997).

31　　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, the affects 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.

9.8.4 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,

1   Westenbarger and Frisvold (1994) measured agricultural exposures to O_3 (and acid precipitation)

2   in the United States. Though not an economic analyses of the costs of ambient exposures, they

3   identified areas of the United States of greatest potential economic damage based on the

4   interface between regional pollution levels and the value of crop production in each region.

5   A study by Murphy et al. (1999) of the economic effects of tropospheric O_3 on U.S.

6   agriculture is of note here, because it confirms the general magnitude of economic effects

7   reported by the two key studies performed a decade earlier (Kopp et al., 1985; Adams, 1986).

8   Specifically, Murphy et al. (1999) evaluated benefits to eight major crops associated with several

9   scenarios concerning the reduction or elimination of O_3 precursor emissions from motor vehicles

10   in the United States. Their analysis reported a $2.8 to 5.8 billion (1990 dollars) benefit from

11   complete elimination of O_3 exposures from all sources, i.e., ambient O_3 reduced to a background

12   level assumed to be 0.025 to 0.027 ppm. While the analytical framework is similar to Adams

13   et al. (1986) in the use of NCLAN-based yield response functions and a mathematical

14   programming-based economic optimization model, the study is novel in its focus on the role of

15   motor vehicle emissions of VOCs/NO_x in total anthropogenic O_3 levels. The study is also

16   notable in its careful attention to federal farm program effects, particularly the deficiency

17   payment component.

18   In addition to these studies in peer-reviewed journals, a number of site-specific effects

19   studies have been performed, primarily by consulting companies for state public utility

20   commissions. Although perhaps of use to public utility commissioners concerned with effects

21   from single power plants or other localized sources, these regional studies generally contribute

22   little to the assessment of air pollution effects at the national level. Also, such reports are not

23   peer reviewed. Therefore, they are not discussed here.

24   There have been a number of recent studies of air pollutant effects on tree species in the

25   literature. Some have reported changes in total biomass and focused on European species

26   (Kurczynska et al., 1997). Other studies have assessed changes in composition of forest species

27   (biodiversity) or forest health due to exposure to air pollutants (Bringmark and Bringmark, 1995;

28   McLaughlin and Percy, 1999; Vacek et al., 1999). As noted previously, changes in forest

29   biomass and composition are more difficult to value than marketable products. However,

30   measures of forest composition or health have implications for an area of increasing policy

31   concern, that being the effect of air pollutants and other environmental stressors on unmanaged

1 (natural) ecosystems and the services they provide (Goulder and Kennedy, 1997; Pimentel et al.,

2 1997). Considerable discussion has occurred among ecologists and economists as to the

3 appropriate means for valuing these services (Anderson, 1990; Carpenter and Dixon, 1985;

4 Common and Perrings, 1992). A number of conceptual articles have been published on this

5 issue in both economic and ecological journals (Bergstrom, 1990; Castle, 1993; Pearce, 1993;

6 Suter, II, 1990).

7　　　　A continuing empirical challenge concerns the lack of information on how changes in

8 biodiversity affect ecosystem performance resulting from O_3 stresses, and the problem of

9 establishing economic values for such changes (Cairnes and Lackey, 1992; Norton, 1987; Pimm,

10 1997; Polasky, 2001; Randall, 1991). As noted in the 1996 AQCD, and more recently by Daily

11 (1999, 2000) and Polasky (2001), there continues to be a lack of empirical studies that actually

12 assess the economic value of changes in biodiversity or in service flows due to any

13 environmental stressors. While some studies report monetary estimates, the estimates are

14 generally for expository purposes and those would not be as defensible as the agricultural studies

15 described earlier. For example, Costanza et al. (1997) assigned a value to the world's

16 ecosystems, but the procedures used render this an exploratory study at best. As assessed by

17 Polasky (2001), "In general, the field of valuation of ecosystem services is in its infancy."

18 He attributed the lack of empirical studies due to both a lack of the understanding of ecology of

19 ecosystem services and to the absence of reliable methods to estimate the value of these services.

20　　　　In summary, the studies of crop and forest responses in the economic literature indicate

21 that O_3 reduces crop yields and imposes economic costs. The economic literature also indicates

22 that O_3 adversely influences the physiological performance of tree species and demonstrates, as

23 expected, that changes in growth have economic consequences. However, the economic data

24 and literature available on ecosystem effects of O_3 exposures are not sufficient to determine the

25 economic costs.

26

27 **9.8.5　Limitations of Scientific Studies and Economic Information**

28　　　　The 1996 O_3 AQCD discussed the need for additional research on both ecological

29 functions and economic methodology in order to better understand the economic implications of

30 air pollutants on ecosystem services. As noted by Daily (1999, 2000), Polasky (2001), and

31 others, this research agenda continues to be important. Despite the large number of discussion

1 and survey articles published since 1996, there still are not sufficient data by which to estimate

2 confidently the magnitude of economic effects of O_3 to forests and natural ecosystems. Nor is it

3 apparent that ongoing research is adequate to answer this question in the next criteria document

4 cycle. Specifically, there does not appear to be any comprehensive, ecological studies underway

5 that attempt to measure changes in ecosystem outputs under alternative O_3 or other air pollutant

6 levels. Thus, in the near term, ecosystem services can only be discussed in qualitative terms.

7 However plausible the likelihood of economic damages to ecosystems, the available scientific

8 and economic information does not provide specific guidance on the magnitudes of these effects.

9   Beside the need to improve our understanding of the effects of O_3 exposures on natural

10 ecosystems, a number of areas of research could help assess the full economic consequences of

11 such pollutants on managed ecosystems. The first of these is the relationship between O_3

12 exposure levels and the variability of crop yields or changes in forest biodiversity. Most

13 assessments are based on the average or expected yield response of a crop to air pollution

14 exposure. However, the variability in yields (the spread or dispersion around the mean) appears

15 to be affected by the nature of plant exposure to pollutants (Hogsett et al., 1985; Musselman

16 et al., 1983). Plants exposed to the same mean dose but with different second moments of the

17 distribution of exposure may have different mean yields (Altshuller and Lefohn, 1996; Lefohn

18 and Benedict, 1982). In addition, the variability of the yield of the plant may also be increased

19 by greater variability in exposures (e.g., a higher frequency of extreme events). The economic

20 significance of higher yield varieties is such that variability may impose additional economic

21 costs, because most farmers have been reported to be averse to risk and prefer less variability for

22 a given yield (or profit). To date, no economic assessments of O_3 damages to agriculture or

23 vegetation in general include risk-averse behavior (studies cited here assumed risk neutrality).

24 To assess the economic consequences of a relationship between farmers' risk preferences and

25 O_3-induced changes in yield variability will require more information on the potential effects of

26 changes in O_3 on crop yield variability. While no economic studies were found on the effects of

27 O_3-induced changes in yield variability, a number of recent studies of climate change effects on

28 crop yields have indicated increased economic costs in the presence of increased climatic

29 variability (e.g., Mearns et al., 1997; Dixon and Segerson, 1999). Analogous economic costs

30 would be expected for changes in air pollution distributions; and these effects need to be

31 examined and quantified.

1   Another research area concerns the need improvement in our understanding of temporal

2   (dynamic) and spatial characteristics of O_3 exposures and their implications for crop yields,

3   production and producer profits. Most economic studies are static, in that they compare two

4   states of the world (e.g., economic activity at one O_3 level versus at an alternative level). In

5   addition, most national- level studies (the type needed to evaluate SNAAQS) display coarse

6   regional-level resolution in terms of crop response, O_3 exposure, and economic behavior. The

7   responses of producers to changes in yields due to changes in O_3 levels are generally assumed to

8   be similar over geographical areas up to several states in size. However, the changes between air

9   quality scenarios are more likely to be characterized by transient changes in exposure levels,

10   which means the producer responses are also likely to be gradual, rather than abrupt. Similarly,

11   the lack of finer scale (regional-level) data and modeling capabilities suggests that important

12   micro-level physical and economic effects are ignored. To what extent these abstractions

13   influence net economic effects is an empirical question. Research on these types of abstractions

14   and assumptions within other economic settings, such as climatic change, have shown that they

15   have implications for economic measurements (Adams, 2002).

16   Another issue is the natural or background level of O_3 (or other pollutants of interest)

17   assumed in economic studies. While many economic studies focus on changes in pollution

18   levels from current conditions, some studies have measured the economic damages between an

19   assumed, or pristine, level and current levels in agricultural areas. Such an analysis, it is

20   reasoned, will provide a measure of the net damages due to anthropogenic sources. The

21   challenge here is to have a correct measure of the background level of the pollutant. Recent

22   research by Lefohn et al. (2001) has suggested that background levels may be considerably

23   higher than assumed in some of the previous economic assessments reported in the 1996 AQCD

24   (25 to 30 ppb in most studies). For example, Lefohn et al. (2001) detected hourly readings of

25   from 40 to 80 ppb during winter and spring months in remote areas of the United States.

26   If background O_3 levels are in the range, then the economic damages estimated in studies with

27   lower background levels will be overstated. The issue of the background O_3 level is important to

28   all assessments of vegetative damages due to O_3.

29   In terms of expanding economic methods for future assessment, analysts should consider

30   using more "reduced form" estimation methods, particularly in situations where the availability

31   of dose-response functions is limited. This estimation approach is exemplified by Garcia et al.

1   (1986). Specifically, their econometric study of the impact of O_3 on producer profits used such a

2   reduced-form approach. In this approach, farmer actions are modeled as a function of ambient

3   O_3 levels (along with other explanatory variables) without the direct use of dose response

4   functions. The advantage of this procedure is that one source of modeling uncertainty, the need

5   for dose-response functions, including time-consuming crop experiments to generate data, is

6   reduced. Actual responses of farmers' profits across air pollution gradients of ambient pollution

7   are observed instead of hypothesized. Although this procedure has not been widely used in air

8   pollution economic assessments, it has been used in a number of relatively recent climate change

9   studies (e.g., Mendelsohn et al. [1994]). The reduced-form method suffers from the fact that if

10   proposed O_3 levels are lower than those observed in the estimation sample, then the prediction

11   accuracy of the method deteriorates. Also, some dose-response information is needed, if only to

12   establish the plausibility of the economic estimates.

13   Another area that may improve economic assessments is incorporating consideration of

14   livestock issues. To date, most agricultural economic assessments of O_3 impacts have ignored

15   the livestock sector. Presumably this is because ambient O_3 levels do not noticeably affect meat

16   yields. However, if feed prices and pasture conditions are affected by ambient O_3 levels, then a

17   more accurate estimate of economic impacts would be forthcoming by including this link to

18   livestock in the assessment. These types of feed production and feed price effects are included

19   in the mathematical programming model used in Adams et al. (1986), but not in most other O_3

20   effects assessments. The significance of livestock-feed linkages are demonstrated in a recent

21   study in the Netherlands by Kuik et al (2000). Using a mathematical programming model

22   similar to that in Adams et al. (1986), Kuik et al. (2000) found that livestock effects were

23   prominent, due mainly to improved pasture yields under reduced ambient O_3 levels.

24

25   ## 9.8.6 Conclusions

26   Substantial progress has been made over the past two decades in our understanding of the

27   effects of O_3 and other oxidants on vegetation, particularly for agriculturally important plant

28   species. The physical and economic effects on agriculture are well documented and provide

29   useful information for the setting of SNAAQS. Effects on forests and natural ecosystems remain

30   problematic, due to limitations in natural science data and economic methods. The problem is

31   most acute for valuing natural ecosystem service flows.

1   The current limitations surrounding forests and natural ecosystems present a rich research

2   agenda. However, not all research needs are likely to lead to better policies. Thus, areas of

3   greatest potential value in terms of regional policymaking need to be prioritized. Such priority

4   setting can be assisted by sensitivity analyses with existing economic models. By measuring the

5   changes in economic effects arising from changes in key parameters, research data gaps most

6   likely to affect economic values can be identified.

7

8

9.9   SUMMARY AND CONCLUSIONS FOR VEGETATION AND ECOSYSTEM EFFECTS

9.9.1   Introduction

12   A significant number of ozone-related studies were published between 1996 and 2004, and

13   they are reviewed in this document in the context of earlier publications reviewed in the previous

14   criteria documents (U.S. Environmental Protection Agency, 1878; 1986; 1992; 1996).

15   In general, there has been a shift away from chamber studies in favor of more field-based

16   approaches although chamber exposures still dominate the effects literature. Field-based

17   approaches include increased survey's of visible injury as well as free-air exposure systems that

18   eliminate some of the problems associated with chamber effects. Increased emphasis has also

19   been placed on quantifying aspects of ozone uptake to better link ambient exposure monitoring

20   with plant/tree response. Much of the progress in quantifying uptake has occurred in Europe

21   with application of models simulating uptake. Evaluation of this new information has added to

22   our knowledge, but has not fundamentally altered the conclusions of previous Ozone Criteria

23   Document (U.S. Environmental Protection Agency, 1996).

24   It is known that ozone is phytotoxic, and that toxicity occurs only if ozone or its reaction

25   products reach the target tissues in the plant cell. Recent studies have provided an increased

26   understanding of how ozone interacts with the plant at the cellular level. Progress has been

27   made on understanding the initial stages of ozone toxicity, which resemble a "wound" response

28   in plants. The alteration of normal metabolism due to a "wound" starts in the cytoplasm, and

29   'spreads' to other cellular organelles. One of the secondary reactions is linked to an activation of

30   a senescence response that results in loss of the critical enzymatic components of photosynthesis.

31   The loss of photosynthetic capacity is in turn linked to the lowered productivity of plants and

1   problems with efficient translocation of carbon. Although photosynthesis and translocation still

2   occurs at a reasonable rate, the loss of productivity, while better understood than in 1996, is still

3   not clearly explained. Dramatic strides in understanding the genetic make-up of plants, gene

4   control, and signal transduction/control over the last few years will accelerate in the future. This

5   understanding will translate into better models, more detailed schemes of how O_3 alters much of

6   the basic metabolism of plants, and how to construct an index that better quantifies exposure and

7   effect. However, the translation of those mechanisms into how O_3 is involved with the altered

8   cell metabolism and subsequent reductions in whole plant productivity and other physiological

9   facts has not yet been fully solved.

10   A number of biotic and abiotic factors are known, or suspected, to alter plant response to

11   ozone. New information published since 1996 does not significantly change the conclusions

12   arrived at in the previous criteria document regarding these multiple interactions. A major

13   concern from the last review was the potential, but unquantified, interaction of ozone and

14   diseases and insects. The nature of these interactions with O_3 are probably dictated by the

15   unique, highly specific biochemical relationships between pathogen and host plant. At this time,

16   therefore, although some diseases may become more widespread or severe as a result of

17   exposure to O_3, it is still not possible to predict which diseases are likely to present the greatest

18   risks to crops and forests.

19   Considerable emphasis during the last decade has been placed on research into O_3

20   interactions with the components of global climate change: increased atmospheric CO_2,

21   increased mean global temperatures, and increased surface level UV-B radiation. To date, the

22   limited experimental evidence and that obtained by computer simulation suggest that even

23   though an enriched CO_2 atmosphere (~600 ppm) would more than offset the impact of O_3 on

24   responses as varied as wheat yield or young Ponderosa growth, the concurrent increase in

25   temperature would reduce but probably not eliminate the net gain. A similar decrease in the net

26   gain resulting from the complete reversal by CO_2 of the inhibition of photosynthesis caused by

27   O_3 has been reported for increased UV-B irradiation. Overall, results are preliminary, based

28   upon minimal data, and do not allow adequate prediction of ozone effects under future climate

29   scenarios. Temperature is unquestionably also an important variable affecting plant response to

30   O_3 in the presence of the elevated CO_2 levels contributing to global climate change. In contrast,

31   evidence continues to accumulate to indicate that exposure to O_3 sensitizes plants to low

1 temperature stress, by reducing belowground carbohydrate reserves, which may lead to

2 responses in perennial species ranging from rapid demise to impaired growth in subsequent

3 seasons.

4   Efforts increased in the area of relating ozone flux (e.g., uptake) to response. Flux is

5 affected by a host of biotic and abiotic factors, including boundary layer conditions, stomatal

6 conductance, moisture conditions, solar irradiance and others. All of these factors provide a

7 more accurate estimation of flux for predicting plant, crop, or forest response. While this area of

8 research is promising, currently there is insufficient data linking flux with response across a

9 range of species or in different ecosystems. In addition, a flux-based standard would require

10 selection of a particular species or species assemblage across a meteorologically similar area in

11 order to adequately model the range of factors known to influence uptake. At this time, there is

12 insufficient information available to employ this approach nationally, but with continued

13 research may eventually provide an alternative index to the current exposure index.

14

9.9.2   Methodology

16   The majority of ozone effects studies are fumigation studies conducted in controlled

17 chambers, as noted in the previous document. The previous document noted that open-top

18 chambers (OTC's) represent the best technology for determination of crop yield to ozone at the

19 present time. While OTC's are still the best method for conducting controlled exposures of

20 varying length and frequency for exposure-response relationships, several new approaches have

21 been applied to ozone research, most notably free-air exposure or FACE systems. FACE

22 systems eliminate many of the concerns raised about closed or open-top chamber experiments,

23 including small plot size, altered microclimate within OTC's, and the effect of charcoal filtering

24 on overall air quality within OTC's. Although FACE systems have increased our understanding

25 in some areas, in most cases results from FACE systems have confirmed what we already

26 understood or hypothesized about how plants and plant assemblages respond to ozone.

27 In particular, studies with aspen showed that ozone symptom expression was generally similar in

28 OTC's, FACE, and also sites along an ambient ozone gradient, supporting the previously

29 observed variation among aspen clones using OTC's (Isebrands et al., 2000; 2001; Karnosky

30 et al., 1999). In addition, root growth is often found to be the most sensitive biomass response to

31 ozone, as reported in the previous document. Caution is still needed, however when making

1 quantitative extrapolations from OTC's, and free-air exposure systems will continue to provide

2 valuable information in support of scaling efforts.

3   The lack of rural monitors continues to be a major problem in the characterization of ozone

4 exposures in remote areas, and in linking effects with exposure in natural ecosystems. Since the

5 last document, the use of passive samplers have expanded monitoring efforts to remote areas that

6 were previously uncharacterized. This has greatly enhanced our ability to link ozone

7 symptomology with elevated exposure in remote areas. Passive samplers do not capture

8 temporal dynamics of exposure, however; therefore, passive samplers are not a substitute for

9 active monitors when attempting to link dynamics of exposure with plant response, or when

10 developing exposure/dose response relationships that are needed in the standard setting process.

11   Exclusion methods such as EDU are the least disruptive of ambient culture conditions in

12 the field, as noted in the previous document. However, the level of protection afforded by EDU

13 is site and species specific, and is subject to local meteorologic conditions. Because of the

14 variability observed in the level of protection provided, and the fact that mechanisms of

15 protection afforded by EDU and other exclusion methods are unknown, caution is needed in

16 applying this approach to the study of ozone effects in the field.

17

18   9.9.3   Mode-of-Action

19   There are several steps in the process of ozone uptake and toxicity that are better

20 understood than in 1996 and are important in developing more refined hypotheses on ozone

21 uptake and mode of action on plants, and developing a flux-based index for use in quantifying

22 response, and ultimately are relevant for developing an air quality standard. Theses are listed

23 below:

24   (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" (Moldau and Bichele, 2002).

25   (2)   The initial reactions of O_3 within the leaf are still unclear but the involvement of
             hydrogen peroxide is clearly indicated. The detection of possible products by EPR
             has progressed but is still not at 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.

26   (3)   The initial sites of membrane reactions seems to be at the 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 due to a change in internal Ca^{2+} levels (Cazale et al., 1998).

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

28   (5)   The alteration of normal metabolism due to a "wound" has affects 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. Certainly the loss of Rubisco and its messenger RNA is linked to an early senescence or a speeding up of normal development leading to senescence. Certainly 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 occurs in a reasonable rate, the loss of productivity is not clearly explained.

29   The new information available on the mode of action of ozone is in part a result of

30 improved molecular tools for following rapid changes that occur within the leaf. Clearly there

31 are many changes that occur within hours or possibly days of the exposure to O_3. However,

32 there is another effect due to O_3 which takes longer to occur and tends to be most obvious under

33 exposure to low O_3 concentrations for long periods. These effects have been linked to

34 senescence or some physiological response very closely linked to senescence (i.e., translocation,

35 reabsorption, allocation of nutrients and carbon).

36   Langebartels et al. (1997) have discussed "memory" or "carry-over effects" within the

37 plant's to explain sensitivity to frost in the winter after exposure to ozone in the summer. Others

38 have argued that it is nutrient status of the tree during the over-wintering phase of its life and

39 chronic exposure to ambient O_3 (less severe with fewer peaks of very high levels) which induces

40 (1) mineral nutrient deficiency; (2) alterations of normal metabolism, including translocation and

41 allocation of carbohydrates and probably nitrogen; (3) disturbance of normal transpiration and

42 diurnal cycling, leading to water stress (Schmieden and Wild, 1995). While general nutrient

43 concentrations within the foliage may not occur, it may be that localized deficiencies do. This is

1   hard to observe or prove without a great deal of work on all portions of a tree and without a

2   general hypothesis of what is occurring.

3   It is important to note that the dramatic strides in understanding the genetic make-up of

4   plants, gene control, and signal transduction/control over the last few years will only accelerate

5   in the future. That understanding will translate into better models of the hypotheses listed and

6   more detailed schemes of how O_3 alters much of the basic metabolism of plants. Thus overall,

7   understanding of how O_3 interacts with the plant at a cellular level has been likewise

8   dramatically improved. However, the translation of those mechanisms into how O_3 is involved

9   with the altered cell metabolism and subsequent reductions in whole plant productivity and other

10   physiological facts has not yet been fully solved. As the understanding of wounding responses

11   of plants and more genome details and varied plant mutants become available, the cellular and

12   physiological responses of plants to O_3 exposures is slowly becoming clearer. However, more

13   studies need to be made on a larger variety of species before this information can be

14   incorporated into indices of response and a protective standard.

15

16   **9.9.4 Modification of Growth Response**

17   There have been only a few studies reported since the 1996 document and none that

18   significantly change the conclusions of the previous criteria document with regard to factors

19   known to alter plant response to ozone.

20   In the area of biotic interactions, new evidence with regard to insect pests and diseases has

21   done little to remove the uncertainties noted in the previous criteria document (see Docherty

22   et al., 1997; and Fluckiger et al., 2002 for recent reviews). Most of the large number of such

23   interactions that may affect crops, forest trees and other natural vegetation have yet to be studied.

24   The trend suggested previously that O_3 increases the likelihood and success of insect attack has

25   received some support from recent studies, but only with respect to chewing insects. With the

26   economically important group of sucking insects such as the aphids, no clear trends have been

27   revealed by the latest studies. Hence, although it seems likely that some insect problems could

28   increase as a result of increased O_3 levels, we are still far from being able to predict the nature of

29   any particular O_3-plant-insect interaction, its likelihood or its severity.

30   The situation is a little clearer with respect to interactions involving facultative,

31   necrotrophic plant pathogens, with O_3 generally leading to increased disease. With obligate,

1   biotrophic fungal, bacterial and nematode diseases, however, there are twice as many reports

2   indicating O_3-induced inhibitions than enhancements. The frequent reports that infection by

3   obligate biotrophs reduces the severity of O_3-induced foliar injury does not result in true

4   "protection" since there are negative effects on the host plant of the disease *per se*. With

5   obligate biotrophs, the nature of any interaction with O_3 is probably dictated by the unique,

6   highly specific biochemical relationships between pathogen and host plant. At this time,

7   therefore, although some diseases may become more widespread or severe as a result of

8   exposure to O_3, it is still not possible to predict which diseases are likely to present the greatest

9   risks to crops and forests.

10   Recent studies have done little to improve our understanding of the nature of interactions

11   between O_3 and root symbionts. Several studies have indicated that the functioning of tree root

12   symbioses with mycorrhizae may be adversely affected by O_3, but there is also evidence that the

13   presence of mycorrhizae may overcome root diseases stimulated by O_3. There is also evidence

14   that O_3 may encourage the spread of mycorrhizae to the roots of uninfected trees.

15   The few recent studies of the impact of O_3 on intra-specific plant competition confirmed

16   that grasses frequently show greater resilience than other types of plants. In grass-legume

17   pastures, the leguminous species suffer greater growth inhibition (Johnson et al., 1996a;

18   Nussbaum et al., 2000a). And the suppression of Ponderosa pine seedling growth by blue wild-

19   rye grass was markedly increased by O_3 (Andersen et al., 2001). However, we are far from

20   being able to predict the outcome of the impact of O_3 on specific competitive situations such as

21   successional plant communities, or crop-weed interactions.

22   Although some recent field studies have indicated that O_3 impact significantly increases

23   with increased ambient temperature, other studies have revealed little effect of temperature.

24   Light, a component of the plant's physical environment, is an essential 'resource' whose energy

25   content drives photosynthesis and CO_2 assimilation. It has been suggested that increased light

26   intensity may increase the sensitivity to O_3 of light-tolerant species, but decrease that of shade-

27   tolerant species, but this appears to be an over-simplification with many exceptions.

28   Temperature affects the rates of all physiological processes based on enzyme-catalysis and

29   diffusion, and each process and overall growth (the integral of all processes) has a distinct

30   optimal temperature range. It is important to note that a plants response to changes in

31   temperature will depend on whether it is growing near its optimum temperature for growth or

1   near its maximum temperature (Rowland-Bamford, 2000). But temperature is unquestionably an

2   important variable affecting plant response to O_3 in the presence of the elevated CO_2 levels

3   contributing to global climate change. In contrast, evidence continues to accumulate to indicate

4   that exposure to O_3 sensitizes plants to low temperature stress, by reducing belowground

5   carbohydrate reserves, which may lead to responses in perennial species ranging from rapid

6   demise to impaired growth in subsequent seasons (i.e., 'carry-over effects').

7   Although the relative humidity of the ambient air has generally been found to increase the

8   adverse effects of O_3 by increasing stomatal conductance and thereby increasing O_3 flux, there is

9   abundant evidence that the ready availability of soil moisture results in greater sensitivity to O_3.

10 The partial 'protection' against the adverse effects of O_3 afforded by drought has been observed

11 in field experiments and modeled in computer simulations (Broadmeadow and Jackson, 2000).

12 There is also compelling evidence that O_3 can predispose plants to drought stress (Maier-

13 Maercker, 1998). Hence the response will depend to some extent upon the sequence in which

14 the stresses occur, but, even though the nature of the responses is largely species-specific,

15 successful applications of model simulations have shown the way to larger scale predictions of

16 the consequences of $O_3 \times$ drought interactions. However, it must be recognized that regardless

17 of the interaction, the net result on growth in the short-term is negative, although in the case of

18 tree species, other responses such as increased water use efficiency could be a benefit to survival

19 in the long term.

20   Somewhat analogously with temperature, it appears that any shift away from the nutritional

21 optimum may lead to greater ozone sensitivity, but the shift would have to be substantial before

22 a significant effect on response to O_3 was observed. Mineral nutrients in the soil, other gaseous

23 air pollutants, and agricultural chemicals constitute chemical factors in the environment. The

24 evidence regarding interactions with specific nutrients is still contradictory: there is some

25 experimental evidence that low general fertility increases sensitivity to O_3, while simulation

26 modeling of trees suggests that nutrient deficiency and O_3 act less than additively, but there are

27 too many example of contrary trends to permit any sweeping conclusions.

28   Interactions of O_3 with other air pollutants have received relatively little recent attention

29 since 1996 (see Barnes and Wellburn, 1998; and Fangmeier et al., 2002 for recent reviews).

30 The situation with SO_2 remains inconsistent, but seems unlikely to pose any additional risk to

31 those related to the individual pollutants. With the NO and NO_2 the situation is complicated by

1   their nutritional value as a source of N. In leguminous species, it appears that NO_2 may reduce

2   the impact of O_3 on growth, with the reverse in other species, but the nature of the exposure

3   pattern, i.e., sequential or concurrent, also determines the outcome. Much more investigation is

4   needed before we will be able to predict the outcomes of different O_3-NO-NO_2 scenarios. The

5   latest research into O_3 × acid rain interactions has confirmed that, at realistic acidities, significant

6   interactions are unlikely. A continuing lack of information precludes offering any

7   generalizations about interactive effects of O_3 with NH_3, HF, or heavy metals. More evidence

8   has been reported for protective effects against O_3 afforded by the application of fungicides.

9   Considerable emphasis during the last decade has been placed on research into O_3

10   interactions with the components of global climate change: increased atmospheric CO_2,

11   increased mean global temperatures, and increased surface level UV-B radiation. However, it

12   must be noted that most of these studies have tended to regard increased CO_2 levels and

13   increased mean temperatures as unrelated phenomena. Experiments into the effects of doubled

14   CO_2 levels at today's mean ambient temperatures are of questionable value in trying to assess the

15   impact of *climate change* on responses to O_3. To date, the limited experimental evidence and

16   that obtained by computer simulation suggest that even though an enriched CO_2 atmosphere

17   (~600 ppm) would more than offset the impact of O_3 on responses as varied as wheat yield or

18   young Ponderosa growth, the concurrent increase in temperature would reduce but probably not

19   eliminate the net gain. A similar decrease in the net gain resulting from the complete reversal by

20   CO_2 of the inhibition of photosynthesis caused by O_3 has been reported for increased UV-B

21   irradiation. However, these are preliminary results based upon minimal data.

22

23   **9.9.5 Exposure Indices**

24   The previous Criteria Document (U.S. Environmental Protection Agency, 1996a) noted

25   that the complexities associated with uptake and ozone interactions with external physical and

26   internal genetic factors that influence plant response makes the development of an 'ideal'

27   exposure index that characterizes plant exposure and response extremely difficult. As a result, a

28   biologically relevant surrogate for uptake was recommended that related ambient exposure to

29   measured growth/yield response. Despite additional research linking estimates of flux with plant

30   response since 1996, there is still insufficient information to identify a flux-based model that

31   incorporates sufficient complexity across space and time to be non-site or non-species specific.

1   Therefore, based on the current state of knowledge, exposure indices that cumulate and

2   differentially weight the higher hourly average concentrations, but include the mid-level values,

3   still represents the best approach in the United States for relating vegetation effects to exposure.

4   The few studies published in this interim have substantiated earlier conclusions on the role

5   of exposure components, including concentration, duration and exposure patterns, in determining

6   the growth response of plants to ozone (Yun and Laurence, 1999; Oksanen and Holopainen,

7   2001). Recent studies using different exposure patterns have confirmed earlier studies on the

8   role of higher concentrations and exposure duration (Nussbaum et. al., 1995). A role for peak

9   concentrations is inferred based on improved air quality in regions like the San Bernardino

10   Mountains in southern California. The reductions of O_3 in the San Bernardino area are

11   associated with reductions in the higher hourly average concentrations, but not in the midrange

12   hourly average concentrations, which are increasing (Lee et al., 2003; Lefohn and Shadwick,

13   2000). General forest improvement is reported following a decrease of O_3 along a decreasing

14   gradient of exposure (Miller and Rechel, 1999; Arbaugh et al., 2003; Tingey et al., 2004). These

15   studies provide the basis for focusing on higher O_3 concentrations, while including the lower

16   levels, when estimating the effects of emission reductions on vegetation.

17   New studies have demonstrated the potential disconnection of peak events and maximal

18   stomatal conductance at xeric to mesic sites in California (Panek et al., 2002; Panek, 2004;

19   Grulke et al., 2002). In addition, a few studies have indicated the uptake of ozone during

20   nighttime hours is greater than previously thought (Grulke et al., 2003; Massman, 2004), and a

21   review of the literature suggests a large number of species exhibit some degree of conductance at

22   night (Musselman and Minnick, 2000). These studies suggest a reconsideration of the need to

23   cumulate exposure 24 hrs per day and not just during daylight hours in exposure index. This

24   lack of coincidence in temporal patterns of conductance and peak ambient concentrations

25   introduces uncertainty in assessing ozone's impact. The use of an exposure index that does not

26   consider regionally unique climate and site factors which modify stomatal conductance may, as a

27   result, under or over estimate growth effects. The shortcomings of an ambient exposure-based

28   index is especially apparent when assessing ozone's potential impact across broad climatic

29   regions of the U.S. or Europe.

30   It is apparent that additional research is needed to develop indices which are more

31   physiologically- and meteorologically-connected to the actual dose of ozone the plant receives.

1   The cumulative, concentration-weighted exposure indices are acknowledged surrogates for

2   effective dose that are conceptually simple and easy to measure. As discussed in the previous

3   Criteria Document, they do not fully characterize the potential for plant uptake and subsequent

4   response since they do not include the physical, biological, and meteorological processes

5   controlling the transfer of O_3 from the atmosphere through the leaf and into the leaf interior.

6   The flux-based approach should provide an opportunity to improve upon the

7   concentration-based (i.e., exposure indices) approach. A great deal of progress has occurred in

8   development and testing of stomatal models that may be generally applicable across certain

9   vegetation types (e.g., Matyssek et al., 2004; Grunhage and Jager 2003; Danielsson et al., 2003;

10   Emberson et al., 2000; Pleijel et al., 2000). While a flux-based approach is preferred, a

11   cautionary argument was advanced in a few publications based on the non-linear relationship

12   between ozone uptake and foliar injury (growth not assessed). The concern is that not all O_3

13   stomatal uptake results in a reduction in yield, which depends to some degree on the amount of

14   internal detoxification occurring with each particular species. Those species having high

15   amounts of detoxification potential may show little relationship between O_3 stomatal uptake and

16   plant response (Musselman and Massman, 1999).

17   The European approach and acceptance of flux-based critical values is in part a recognition

18   of the problems associated with ambient exposure-based indices. Research continues across

19   Europe to develop the necessary experimental database and modeling tools that will be required

20   to provide the scientific basis for a critical level for ozone (Grunhage et al., 2004; Fuhrer et al.,

21   1997; Grunhage and Jager, 1994).

22   Given the current state of knowledge and the best available data, exposure indices that

23   cumulate and differentially weight the higher hourly average concentrations, but include the

24   mid-level values, continue to offer the most defensible approach for vegetation protection.

25   A large database exists that has been used for establishing exposure-response relationships, and

26   at this time, such a database does not exist for relating ozone flux to growth response.

27   It is anticipated that as the overlapping relationships of conductance, concentration, and

28   defense mechanisms are better defined, the flux-based indices may be able to predict vegetation

29   injury and/or damage with more accuracy than the exposure-response models. However, it is

30   unclear that such is the case at this time. The translation of these research and assessment tools

1   to air quality standards has the additional need to be simple, understandable, and adaptive to a

2   manageable monitoring program.

3

9.9.6 Ozone Exposure-Plant Response Relationships

5   Data published since 1996 continue to support the conclusions of previous criteria

6   documents that there is strong evidence that ambient concentrations of O_3 cause foliar injury and

7   growth and yield damage to numerous common and economically valuable plant and tree

8   species. For annual vegetation, the data summarized in Table 4-19 show a range of growth and

9   yield responses both within species and among species. Nearly all of these data were derived

10 from studies in OTCs, with only two studies using open-air systems in the UK (Ollerenshaw

11 et al., 1999; Ollerenshaw and Lyons, 1999). It continues to be difficult to compare studies that

12 report O_3 exposure using different indices, such as AOT40, SUM06, or 7-h or 12-h mean values.

13 However, when such comparisons can be made, the results of recent research confirm earlier

14 results summarized in the previous criteria document (U.S. Environmental Protection Agency,

15 1996). A summary of earlier literature concluded that a 7-h 3-month mean of 49 ppb

16 corresponding to a SUM06 exposure of 26 ppm·h, would cause 10% loss in 50% of 49

17 experimental cases (Tingey et al., 1991). Recent data summarized in Table 4-19 support this

18 conclusion, and more generally indicate that ambient ozone exposures can reduce the growth and

19 yield of annual species. Some annual species such as soybean are more sensitive, and greater

20 losses may be expected (Table 4-19). Thus the recent scientific literature supports the

21 conclusions of the previous criteria document that ambient O_3 concentrations are reducing the

22 yield of major crops in the United States.

23   Much research in Europe has used the AOT40 exposure statistic, and substantial effort has

24 gone into developing Level-1 critical levels for vegetation using this index. Based on regression

25 analysis of 15 OTC studies of spring wheat including one study from the U.S. and 14 from

26 locations ranging from southern Sweden to Switzerland, an AOT40 value of 5.7 ppm·h was

27 found to correspond to a 10% yield loss, and a value of 2.8 ppm·h corresponded to a 5% yield

28 loss (Fuhrer et al., 1997). Because a 4 to 5% decrease could be detected with a confidence level

29 of 99%, a critical level of an AOT40 value of 3 ppm·h was selected in 1996 (Kärenlampi and

30 Skärby, 1996).

1   In addition to reductions in crop yield, O_3 may also reduce the quality or nutritive value of

2   annual species. Many studies have shown effects of O_3 on various measures of plant organs that

3   affect quality, with most studies focusing on characteristics important for food or fodder. These

4   studies indicate that there may be economically important effects of ambient O_3 on the quality of

5   crop and forage species. Previous criteria documents have concluded that visible symptoms on

6   marketable portions of crops and ornamental plants can occur with seasonal 7-h mean O_3

7   exposures of 40 to 100 ppb (U.S. Environmental Protection Agency, 1978; 1986; 1996). The

8   recent scientific literature does not refute this conclusion.

9   The use of OTCs may reverse the usual vertical gradient in O_3 that occurs within a few

10   meters above the ground surface (Section 4.2). This reversal suggests that OTC studies may

11   overestimate to some degree the effects of an O_3 concentration measured several meters above

12   the ground. However such considerations do not invalidate the conclusion of the previous

13   criteria document that ambient O_3 exposures are sufficient to reduce the yield of major crops in

14   the United States.

15   As for single-season agricultural crops, yields of multiple-year forage crops are reduced at

16   ozone exposures that occur over large areas of the U.S. This result is similar to that reported in

17   the previous criteria document (U.S. Environmental Protection Agency, 1996). When species

18   are grown in mixtures, O_3 exposure can increase the growth of tolerant species while

19   exacerbating the growth decrease of O_3-sensitive species. Because of this competitive

20   interaction, the total growth of the mixed-species community may not be affected by O_3

21   exposure. However, in some cases mixtures of grasses and clover species have shown

22   significant decreases in total biomass growth in response to O_3 exposure in studies in the U.S.

23   and in Sweden. In Europe, a provisional critical level for herbaceous perennials of an AOT40

24   value of 7 ppm·h over six months has been proposed to protect sensitive plant species from

25   adverse effects of O_3.

26   For deciduous tree species, recent evidence from free air exposure systems and OTCs

27   supports results observed previously in OTC studies. For example, a series of studies was

28   undertaken using free air O_3 enrichment in Rhinelander, WI (Isebrands et al., 2000; 2001).

29   These studies showed that O_3 symptom expression was generally similar in OTCs, FACE, and

30   also sites along an ambient O_3 gradient, supporting the previously observed variation among

1   aspen clones using OTCs (Karnosky et al., 1999). As has been observed in previous criteria

2   documents, root growth often is found to be the most sensitive biomass response to O_3.

3   Results since 1996 support the conclusion of the previous criteria document (U.S.

4   Environmental Protection Agency, 1996) that deciduous trees are generally less sensitive to

5   O_3 than are most annual plants, with the exception of a few very sensitive genera such as

6   *Populus* and sensitive species such as black cherry. However, the data presented in Table 4-20

7   suggest that ambient exposures that occur in the U.S. can sometimes reduce the growth of

8   seedlings of deciduous species. Results from multiple-year studies sometimes show a pattern of

9   increased effects in subsequent years. In some cases however, growth decreases due to O_3 may

10 become less significant or even disappear over time. While some mature trees show greater O_3

11 sensitivity than do seedlings in physiological parameters such as net photosynthetic rate, these

12 effects may not translate into measurable reductions in biomass growth. However, because even

13 multiple-year experiments do not expose trees to O_3 for more than a small fraction of their life

14 span, and because competition may in some cases exacerbate the effects of O_3 on individual

15 species, determining effects on mature trees remains a significant challenge.

16   In Europe, a Level I critical level has been set for forest trees based on OTC studies of

17 European beech seedlings. A critical level was defined as an AOT40 value of 10 ppm·h for

18 daylight hours for a 6-month growing season (Kärenlampi and Skärby, 1996). However, other

19 studies show that other species such as silver birch may be more sensitive to O_3 than is beech

20 (Pääkkönen et al., 1996).

21   For evergreen tree species as for other tree species, the O_3 sensitivity of different genotypes

22 and different species varies widely. Based on studies with seedlings in OTCs, major species in

23 the US are generally less sensitive than are most deciduous trees, and slower growing species are

24 less sensitive than are faster growing species. There is evidence that interacting stresses such as

25 competition may increase the sensitivity of trees to O_3. As for deciduous species, most

26 experiments with evergreen species have only covered a very small portion of the life span of a

27 tree, and have been conducted with seedlings, so estimating effects on mature trees is difficult.

28   For all types of perennial vegetation, cumulative effects over more than one growing

29 season may be important, and studies for only a single season may underestimate effects.

30 Mature trees may be more or less sensitive to O_3 than are seedlings depending on the species,

31 but information on physiological traits can be used to predict such differences in some cases.

1   In some cases, mature trees may be more sensitive to O_3 than seedlings due to differences in gas

2   exchange rates, differences in growth rates, greater cumulative exposure, or the interaction of

3   ozone with other stresses.

4

5   **9.9.7 Ecosystem Effects**

6   There is evidence that tropospheric O_3 is an important stressor of ecosystems, with

7   documented impacts on the biotic condition, ecological processes, and chemical/physical nature

8   of natural ecosystems. In turn, the effects of O_3 on individual plants and processes are scaled up

9   through the ecosystem affecting processes such as energy and material flow, inter- and intra-

10   species competition, and NPP. Thus, effects on individual keystone species and their associated

11   microflora and fauna, which has been shown experimentally, may cascade through the

12   ecosystem to the landscape level, although it has not yet been demonstrated. By affecting water

13   balance, cold hardiness, tolerance to wind, and by predisposing plants to insect and disease pests,

14   O_3 may even impact the occurrence and impact of natural disturbance (e.g., fire, erosion).

15   Despite the probable occurrence of such effects, there are essentially no instances where

16   ecosystem level, highly integrated studies have conclusively shown that ozone is indeed altering

17   ecosystem structure and/or function.

18   Systematic injury surveys demonstrate that foliar injury occurs on sensitive species in

19   many regions of the USA. However, the frequent lack of correspondence between foliar

20   symptoms and growth effects means that other methods must be used to estimate the regional

21   effects of O_3 on tree growth rates. Investigations of the radial growth of mature trees in

22   combination with data from many controlled studies with seedlings and a few studies with

23   mature trees suggest that ambient O_3 is reducing the growth of mature trees in some locations.

24   Studies using models based on tree physiology and forest stand dynamics suggest that modest

25   effects of O_3 on growth may accumulate over time, and may interact with other stresses. For

26   mixed-species stands, such models predict that overall stand growth rate is generally not likely to

27   be affected. However, competitive interactions among species may change as a result of growth

28   reductions of sensitive species. These results suggest that O_3 exposure over decades may be

29   altering the species composition of forests in some regions.

30   Despite increased understanding of possible ecosystem effects of ozone, the data base

31   demonstrating and quantifying the degree to which O_3 is altering natural ecosystems is lacking.

1   Much of the speculation of ozone impact on ecosystems must be inferred from a number of case

2   studies of forest plot field-based data reporting on a number of different species. One means to

3   discuss our current knowledge is by listing the areas in which there is lack of knowledge. These

4   include:

5   *Ecosystem processes*. Very little is known about the effects of O_3 on water, carbon, and

6   nutrient cycling, particularly at the stand and community levels. Effects on belowground

7   ecosystem processes in response to O_3, exposure alone and in combination with other stressors

8   are critical to projections at the watershed and landscape scales. Little is yet known about the

9   effects of O_3 on structural or functional components of soil food webs or how these impacts

10   could affect plant species diversity (Andersen, 2003).

11   *Biodiversity and genetic diversity*. The study of genetic aspects of O_3 impacts on natural

12   ecosystems has been largely based on correlations and it remains to be shown conclusively

13   whether O_3 affects biodiversity or genetic diversity (Pitelka, 1988; Winner et al., 1991; Davison

14   & Barnes, 1998). Studies of competitive interactions under elevated O_3 are needed (Laurence &

15   Andersen, 2003), and re-examination via new sampling of population studies to bring in a time

16   component to previous studies showing spatial variability in population responses to O_3 are

17   needed. These studies could be strengthened by modern molecular methods to quantify impacts

18   on diversity.

19   *Natural ecosystem interactions with the atmosphere*. Little is known about feedbacks

20   between O_3 and climate change on production of volatile organic compounds, which in turn,

21   could affect O_3 production (Fuentes et al., 2001). At moderate to high O_3 exposure sites,

22   aberrations in stomatal behavior could significantly affect individual tree water balance of

23   sensitive trees, and if the sensitive tree species is dominant, hydrologic balance at the watershed

24   and landscape level could be affected. This has not been addressed in any model because O_3

25   exposure effects, if included at all in the modeling effort, have assumed a linear relationship

26   between assimilation and stomatal conductance. Interaction studies with other components of

27   global change (i.e., warming, increasing atmospheric CO_2, N deposition, etc.) or with various

28   biotic stressors are needed to better predict complex interactions likely in the future (Laurence &

29   Andersen, 2003). Whether O_3 will negate the positive effects of an elevated CO_2 environment

30   on plant carbon and water balances is not yet known nor is it known if these effects will scale-up

1   through the ecosystem. How O_3 affects the progress of pest epidemics and insect outbreaks as

2   concentrations increase is unclear (Skarby et al., 1998).

3   Information concerning the impact of O_3 on reproductive processes and reproductive

4   development under realistic field or forest conditions are needed as well as examination of

5   reproductive effects under interacting pollutants (Black et al., 2000).

6   *Scaling*. The vast majority of O_3 studies of trees have been conducted with young,

7   immature trees and in trees that have not yet formed a closed canopy. Questions remain as to the

8   comparability of O_3 effects on juvenile and mature trees and on trees grown in the open versus

9   those in a closed forest canopy in a competitive environment (Chappelka & Samuelson, 1998;

10 Kolb & Matyssek, 2001; Samuelson & Kelly, 2001). Scaling the effects of O_3 across spatial

11 scales is also difficult. Scaling responses of single or a few plants to effects on communities and

12 ecosystems are complicated matters that will require a combination of manipulative experiments

13 with model ecosystems, community and ecosystem studies along natural O_3 gradients, and

14 extensive modeling efforts to project landscape level, regional, national and international

15 impacts of O_3. Linking these various studies via impacts on common research quantification

16 across various scales using measures of such factors as leaf area index or spectral reflective data,

17 which could eventually be remotely sensed (Kraft et al., 1996; Panek et al., 2003), would provide

18 powerful new tools for ecologists.

19   *Identifying endpoints*. In general, methodologies to determine the important values of

20 services and benefits derived from natural ecosystems are lacking. Identifying and quantifying

21 factors that could be used in comprehensive risk assessment for O_3 effects on natural ecosystems

22 would increase societal awareness of the importance of protecting ecosystems (Heck et al.,

23 1998).

24

9.9.8 Economics

26   Substantial progress has been made over the past two decades in our understanding of the

27 effects of ozone and other oxidants on vegetation, particularly for agriculturally-important plant

28 species. The physical and economic effects on agriculture are well documented and provide

29 useful information for the consideration of establishing air quality standards for crops. Effects

30 on forests and natural ecosystems remain problematic, due to limitations in biological response

1 data and economic methods. The problem is even more acute for valuing natural ecosystem

2 service flows.

3   The current limitations surrounding forests and natural ecosystems present a rich research

4 agenda. However, not all research needs are likely to lead to better policies. Thus, areas of

5 greatest potential value in terms of regional policy making need to be prioritized. Such

6 priority-setting can be assisted by sensitivity analyses with existing economic models; by

7 measuring the changes in economic effects arising from changes in key parameters, it is possible

8 to identify those research data gaps most likely to affect economic values.

9

10

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10. TROPOSPHERIC OZONE EFFECTS ON UV-B FLUX AND CLIMATE CHANGE PROCESSES

10.1 INTRODUCTION

In addition to exerting direct effects on human health and vegetation/ecosystems, tropospheric ozone (O_3) is involved in determining ground-level flux of solar ultraviolet (UV) radiation and, also, in other processes that alter the earth's radiative balance and contribute to resulting climate change. This chapter first discusses the role of tropospheric ozone in determining surface-level UV flux and, then, secondly, discusses tropospheric O_3 involvement in global climate change.

10.2 THE ROLE OF TROPOSPHERIC OZONE IN DETERMINING GROUND-LEVEL UV-B FLUX

Stratospheric O_3 plays a crucial role in reducing the exposure of living organisms to solar ultraviolet radiation. The stratosphere, the atmospheric layer immediately above the troposphere, is roughly 50 km thick and possesses 90% or more of the atmosphere's O_3. Ozone depletion due to the release of long-lived anthropogenic chlorinated and fluorinated hydrocarbons was discovered over the course of the 1970s and early 1980s and led to establishment of an international treaty for the protection of stratospheric O_3, the 1987 Montreal Protocol on Substances that Deplete the Ozone Layer.

Active research has been underway into the effects of increased UV radiation on ecosystems and on human health since the discovery of the seasonal polar "Ozone Hole" and the declining stratospheric O_3 concentrations in the midlatitudes. An outcome of this effort is a body of literature that also describes the effects of tropospheric pollutants, PM, and O_3, on ground-level UV radiation flux. The Montreal Protocol requires routine review of the latest scientific information available on the status of the O_3 layer and of UV radiation levels at the earth's surface. The World Meteorology Organization (WMO) and UN Environmental Program (UNEP) are responsible for assessing the state of the science regarding the O_3 layer and for

1   reporting on trends in surface UV radiation levels. The latest WMO/UNEP assessment was

2   published in 2003 (WMO/UNEP, 2002).

3   This section describes the current level of scientific understanding of the factors

4   influencing the flux of UV radiation at the earth's surface, including geophysical factors,

5   tropospheric O_3, PM, and cloud cover, with reference to the WMO/UNEP assessment and the

6   peer-reviewed literature. Factors influencing the degree of human exposure to UV-B and the

7   resulting health effects are also discussed.

8

9   ## 10.2.1   Factors Governing Ultraviolet Radiation Flux at the Earth's Surface

10   ### 10.2.1.1   UV Radiation: Wavelengths, Energies and Depth of Atmospheric Penetration

11   Designations for portions of the electromagnetic spectrum have evolved over time and are

12   usually associated with general function or effects caused by photons in a given wavelength

13   range. Gamma rays, at or below 0.1 nm in wavelength, are especially damaging high energy

14   photons emitted during radioactive decay and by stellar activity. Radiowaves, wavelengths

15   greater than 10^8 nm, are very low in energy and function as carriers for broadcast

16   communications. The energy possessed by any photon is inversely proportional to its

17   wavelength.

18   The wavelengths ranging between 50 and 400 nm in length are denoted "ultraviolet."

19   Solar radiation of wavelengths < 280 nm, including UV-C (200 to 280 nm), is almost entirely

20   blocked by Earth's upper atmosphere due to photoionization and photo-dissociation processes.

21   Figure 10-1 shows a comparison between solar flux above the atmosphere and the flux at the

22   earth's surface. The flux of solar radiation between 280 and 320 nm (UV-B) is absorbed or

23   scattered in part within the atmosphere, while radiation between 320 and 400 nm (UV-A) can be

24   scattered but are not absorbed by gases to any meaningful degree. UV-B and UV-A photons

25   contain the necessary levels of energy needed to break (photolyze) chemical bonds. Both UV-A

26   and UV-B are associated with human health- and ecosystem-damaging effects. Because UV-B

27   is more energetic, it is potentially capable of producing more biological damage than UV-A.

28   UV-A comprises 90% of the UV radiation that reaches the earth's surface, yet is 1000 times less

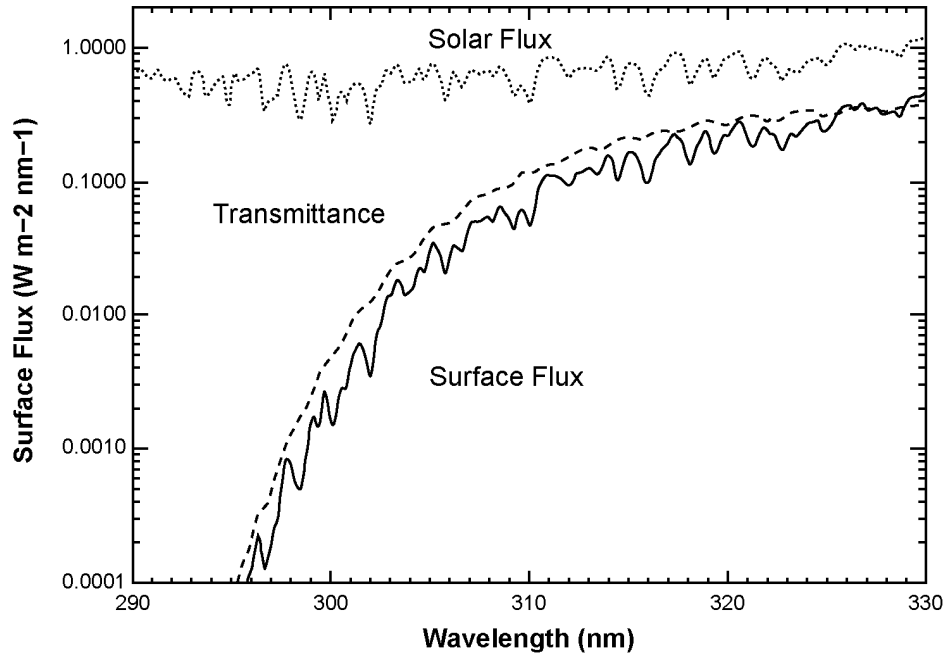29   damaging to keratinocytes (skin cells), than UV-B (Hildesheim, 2004).

30

31

Figure 10-1. **Extraterrestrial solar flux measured by the satellite UARS SOLSTICE instrument (dotted line). The dashed line represents calculated atmospheric transmittance and the solid line is the calculated absolute flux of UV radiation for a solar zenith angle of 50deg, total column O3 of 275 DU, and a surface reflectivity of 8%. The fine structure on the surface flux trace results from Fraunhofer lines (absorption specific wavelengths within the solar atmosphere).**

Source: Krotkov et al. (1998).

10.2.1.2 Temporal Variations in Solar Flux

The magnitude of the solar radiation flux entering the atmosphere is dependent upon long-term solar activity, sunspot cycle (11 years), solar rotation (27 days) and the position of the earth in its orbit around the sun. A variety of changes in solar irradiance can be found in the historical data, beginning in 1700 and leading up to the present. Fligge and Solanki (2000) concluded that solar irradiation changes on time scales of days to centuries can be attributed to variations in solar magnetic features. Since the last Maunder minimum in 1700, solar irradiance has increased very slightly, at approximately 3.0% for wavelengths < 300 nm and at ~1.3% for the UV-B and UV-A range. Including visible wavelengths, Fligge and Solanki (2000) estimated that the overall increase in solar irradiance was ~0.3%. Rozema et al. (2001) pointed out that any

1   increase in wavelengths < 300 nm (UV-C) would initiate additional O_3 formation in the

2   stratosphere, therefore increasing its UV radiation absorptive capacity.

3   Solar rotation and sunspot activity have the greatest effect on the radiation flux that

4   originates in the highest levels of the solar atmosphere. The amplitude of the associated cyclical

5   changes in solar shortwave radiation flux follows an inverse relationship between the photon's

6   wavelength and the solar altitude at which it was emitted. The maximum level of radiation

7   (solar-max) differs from the minimum (solar-min) by as much as 10% for wavelengths near

8   160 nm. This peak-to-trough difference declines to around 1% for 300 nm (UV-B range)

9   (Salby, 1996).

10   The combined effects of the earth's obliquity (the angle of the earth's axis of rotation with

11 respect to the plane of its orbit around the sun) and its precession (the rotation of the earth's axis

12 with respect to a perpendicular line through the plane of its solar orbit) yield variations of up to

13 30% in total summertime solar flux, depending on latitude (Hartmann, 1994).

14

15 *Zenith Angle: Latitude, Season and Time of Day*

16   The sun's relative elevation is measured with respect to the vertical and is known as its

17 "zenith angle." This angle varies hourly, seasonally, and with latitude. Daily and seasonal

18 changes in solar zenith angle result in the largest changes in the magnitude of solar radiation

19 flux, with higher zenith angles corresponding to lower solar flux. The largest natural fluxes

20 occur in the tropical regions, where solar noon occurs at a zenith angle at or near 0°. Seasonal

21 variation in solar flux ranges from small changes at the equator to very large changes at high

22 latitudes. Daily variations in solar flux, from sunrise to sunset, show added wavelength

23 dependence as a function of zenith angle, because transmission of some wavelengths are

24 sensitive to atmospheric pathlength due to scattering and absorption processes. These processes

25 will be discussed further below.

26

27 **10.2.1.3 Atmospheric Radiative Interactions with Solar UV Radiation**

28 ***Radiative Interactions in the Stratosphere***

29   The stratosphere contains 90% or more of the total column density of O_3, the principle gas

30 phase absorber of UV-B. Ozone interacts with UV radiation by scattering the photon or

31 absorbing and transforming its energy. Upon absorbing a UV photon, O_3 may photodissociate,

32 or become electronically- and vibrationally-excited.

1   Photoabsorption by O_3 occurs with very high efficiency. After electronically-excited O_3

2   (O_3*) is formed, it will either dissociate into ground-state oxygen, O_2, and an electronically

3   excited oxygen radical, $O(^1D)$, or a collision between the O_3 molecule with another gas molecule

4   (M) may occur (See Reactions 1 and 2). Intermolecular collisions degrade the excess electronic

5   energy of the O_3 molecule by transferring it to other molecules as vibrational, rotational, and/or

6   translational energies, which warm the atmosphere. The electronically excited oxygen radical

7   can then react with H_2O to form two hydroxyl radicals, OH (Reaction 3). See Chapter 2 for

8   further discussion of odd oxygen and HO_x photochemistry.

$$O_3 + h\nu \rightarrow O_3^* \rightarrow O(^1D) + O_2 \qquad\qquad (10\text{-}1)$$

$$\rightarrow O_3^* + M \rightarrow O_3 + M^* \qquad\qquad (10\text{-}2)$$

9

10

$$O(^1D) + H_2O \rightarrow 2OH \qquad\qquad (10\text{-}3)$$

11

12

13   Either of these photochemical processes transforms the energy of the UV photon into a form

14   lacking the potential for human health or ecosystems damage.

15   The WMO/UNEP (2002) scientific assessment reported that global average total column

16   O_3 had declined by 3% from pre-1980 levels, due to the presence of anthropogenic ozone-

17   depleting substances in the atmosphere. Ozone depletion has a strong latitude and seasonal

18   dependence. The seasonality of total O_3 changes differ between the Northern and Southern

19   Hemispheres. In the northern midlatitudes, total column O_3 declined by ~4% during the

20   winter/spring seasons and by approximately half that amount in the summer/fall of the

21   1997-2001 time period, relative to pre-1980 total column O_3 levels. In southern midlatitudes,

22   total column O_3 declined ~6% during all seasons.

23   The concentration of O_3 in a vertical column extending from the earth's surface is

24   expressed in Dobson Units (DU), corresponding to the column height in hundredths of a

25   millimeter of O_3 at standard temperature and pressure (273 K and 1 atmosphere) (Wayne, 2000).

26   One DU $= 2.587 \times 10^{15}$ molecules of O_3/cm^2. The total O_3 column effectively prevents any

27   UV-C from reaching the surface, reduces the penetration of UV-B to the surface but does little to

1   attenuate the intensity of UV-A except at the shorter wavelengths close to the cut off for UV-B.

2   Cutchis (1974) calculated that with overhead sun a 10% decrease in the O_3 column would lead to

3   20, 250, and 500% increases in flux at 305, 290, and 287 nm, respectively, values that have been

4   supported by ground observations in Toronto, ON (49° N; Kerr and McElroy, 1993). Rapid

5   changes of this magnitude appear to happen naturally. As seen in data collected by the Total

6   Ozone Mapping Satellite (TOMS) (Figure 10-2), the total O_3 column undergoes wide natural

7   variation on short timescales (Cockell, 2001).

8

9

Ozone Column (1990-1992)
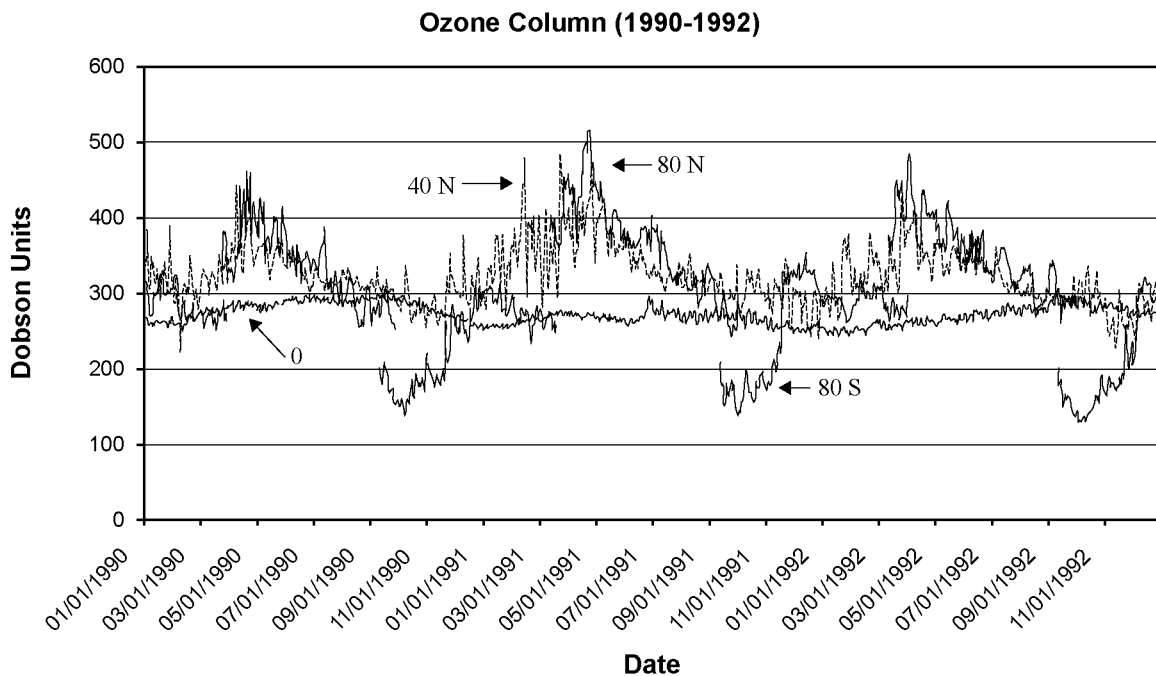
**Figure 10-2.   Ozone column abundances from the years 1990 to 1992 for 0, 40, and 80° N
as well as 80° S. The data for 80° S are incomplete, but the graph shows the
effects of the Antarctic O_3 hole on total column abundances at this latitude.
The data for the Northern Hemisphere illustrate the natural variations in the
O_3 column over time. The data are taken from the TOMS (Total Ozone
Monitoring Satellite) data set (1979 to 1993).**

Source: Cockell (2001).

1   Nacreous and polar stratospheric clouds scatter radiation and aerosols, such as those

2   injected into the stratosphere by explosive volcanic eruptions, both absorb and scatter radiation.

3   Relative to the troposphere, the stratosphere is low in atmospheric pressure. Stratospheric clouds

4   and aerosols are also more dispersed than in the troposphere. Consequently, UV radiation can

5   traverse the stratosphere with a substantially lower probability of encountering a gas molecule or

6   cloud or aerosol particle than it would in the troposphere. In the radiative transfer literature, the

7   stratosphere is described as a "single scattering" regime for UV radiation, and UV that has

8   penetrated the stratosphere is referred to as "direct beam UV." The troposphere, due to its high

9   gas and particle concentrations is referred to as a "multiple scattering" regime.

10

11   ***Radiative Interactions in the Troposphere: Solar Irradiance vs. Actinic Flux***

12   The troposphere contains $\leq 10\%$ of the total column O_3 but ~78% of the total atmospheric

13   mass including clouds, gas- and particle-phase radiation scatterers and absorbers, making it a

14   "multiple scattering" regime for UV radiation. These scattering processes increase the mean-

15   free path a photon must travel before reaching the surface, transforming the direct beam UV

16   solar irradiance that has penetrated the stratosphere into diffuse, or actinic, UV (Brühl and

17   Crutzen, 1989).

18   Atmospheric scattering processes, such as Rayleigh and Mie scattering, are particle-size

19   dependent. In Rayleigh scattering, gas molecules that are smaller than the wavelength of the

20   incident photon isotropically deflect incoming photons. With Mie scattering, aerosol and cloud

21   droplets scatter incoming radiation with forward- and back-scattering tendencies. Actinic flux,

22   especially at the earth's surface, is directly proportional to surface albedo (Wendisch and Mayer,

23   2003). Surface albedo is very strongly wavelength dependent. For example, fresh and wet snow

24   reflects 60 to 90% of violet light, while soil and grass surfaces reflect < 5% of incident violet

25   light (Xenopoulos and Schindler, 2001). Wendisch and Mayer (2003) found, in their in situ

26   measurement and modeling study of the vertical distribution of solar irradiance, that surface

27   albedos must be measured in order to accurately simulate solar flux, due to possibly large

28   variations in albedo within a given surface type. Snow cover, even many kilometers from

29   measurement sites is known to increase detected UV irradiances. Complicated interactions

30   result when radiation is scattered by snow (or other bright surfaces) and backscattered or

31   absorbed by atmospheric particles and clouds in the same vicinity (WMO/UNEP, 2002).

1   *Variation in Solar Flux with Altitude*

2   Solar flux increases with altitude above sea level, due to the decreased presence of clouds

3   and declining concentrations of scattering and absorbing atmospheric pollutants. Rayleigh

4   scattering, discussed below, also lessens with decreasing atmospheric pressure. A number of

5   measurements of UV radiation have been taken as a function of altitude and are reviewed by

6   Xenopoulos and Schindler (2001). Increases in flux as a function of altitude are given as percent

7   irradiance enhancement per 1000 m relative to sea level. The effect can range from 9 to 24%

8   /1000 m as function of the altitude at which the measurement was taken (Xenopoulos and

9   Schindler, 2001). The effect corresponds to the relative pathlength traveled by the solar photon:

10   flux is strongest when the photon is not impeded by atmospheric scattering or absorbing agents.

11   Similarly, this effect is seen as a function of solar zenith angle, i.e., flux is at its maximum when

12   the atmospheric depth through which the photon must pass is at its shallowest.

13

14   *Clouds*

15   In principle, clouds have the largest influence on surface level UV irradiance, but their

16   effects are difficult to quantify. The depth and composition of a cloud determines, in part, the

17   amount and wavelengths of radiation that it will scatter or absorb. Geometry is an especially

18   important factor, as scattered or broken clouds may enhance, rather than reduce, surface flux.

19   For example, if the sun is not fully blocked by a cloud, the reduction in irradiance may be small

20   (WMO/UNEP, 2002). Quantifying the effect of clouds on surface UV flux, therefore, requires

21   information on cloud composition, geometry, altitude, and the position of the sun relative to the

22   cloud and the underlying surface, as a function of time. Provided that all of this detailed

23   information is available, a three dimensional model is then required to calculate surface-level

24   reductions or enhancements in UV flux.

25

26   *Particulate Matter*

27   On a zonally averaged basis, PM does not contribute significantly to lower tropospheric

28   absorption of UV radiation. However, in urban areas or other areas subject to high smog levels

29   (areas of significant biomass combustion), PM may be the most important determinant of

30   ground-level erythemal UV flux, second only to cloud cover (U.S. Environmental Protection

31   Agency, 2004; WMO/UNEP, 2003). Model-to-measurement comparisons of ground-level flux

1   for Greece and Toronto have shown 20 and 5-10% reductions, respectively (McKenzie et al.,

2   2001). Increases over the past 20 to 30 years in combustion-associated PM and black carbon

3   may account for the inability to detect a surface trend in UV-B radiation caused by a known

4   decrease in stratospheric O_3 over the Northern Hemisphere (Barnard et al., 2003).

5

6   ***Gases***

7   In the upper troposphere, the UV-absorbing gases O_3 and, of lesser importance,

8   formaldehyde (CH_2O) and SO_2 are vented or diffuse from the surface. Stratospheric intrusions

9   extrude O_3-rich air into the troposphere where it mixes, increasing regional background O_3 levels

10   (see Chapter 3). Tropospheric O_3 data are typically expressed on a concentration basis, e.g.,

11   parts per billion by volume (ppbv), where 1 ppbv tropospheric $O_3 \equiv 0.65$ DU (IPCC, 2001). The

12   mean values of O_3 in the free troposphere reported in the literature range from ~50 to ~80 ppbv,

13   with higher values occurring at the tropopause. For example, a series of ozonesonde soundings

14   over France during the period from 1976 to 1995 showed an O_3 increase from 48.9 ppbv in the

15   2.5 to 3.5 km layer to 56.5 ppbv in the 6.5 to 7.5 km layer, although the data revealed no

16   significant increasing trend over time (Ancellet and Beekmann, 1997).

17   Close to the surface in polluted urban settings, photochemistry produces a diurnal rise and

18   fall in O_3 and PM concentrations. Temperature inversions tend to prevent the upward mixing

19   and dilution of ground-level O_3, while trapping primary and secondary PM. A recent study of

20   the concurrence of O_3 and PM is provided by Koloutsou-Vakakis et al. (2001). No measurement

21   technique is currently available that can distinguish between absorption of incident UV radiation

22   by O_3 versus absorption by PM.

23   UV absorption by gases becomes important under aerosol- and cloud-free conditions.

24   Figure 10-3 shows a calculation by Krotkov et al. (1998) of the sensitivity, as a function of

25   wavelength, of ground-level UV flux to a 1-DU decrease in total column O_3 under cloud- and

26   aerosol-free conditions. A 1991 to 1992 study in Chicago in which ambient O_3, broadband UV

27   irradiance and total sunlight were monitored (Frederick et al., 1993), produced a statistically

28   significant negative correlation between the UV irradiance and ambient O_3 when the atmosphere

29   was relatively free of clouds and haze. Although they estimated that a 10-ppbv reduction in O_3

30   was associated with a 1.3% increase in erythemally-weighted UV-B, they cautioned that this

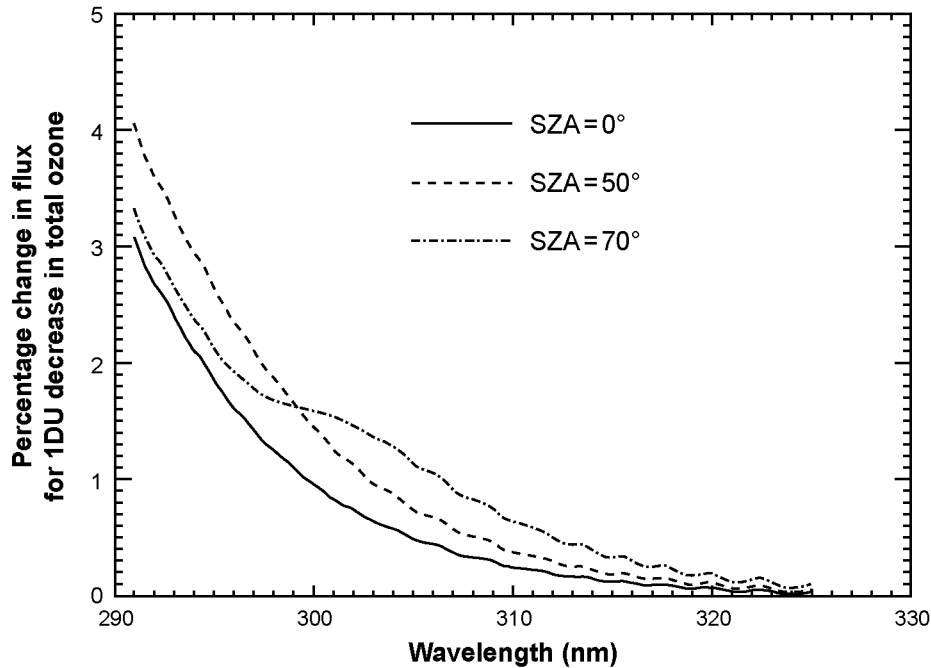31   figure had a comparatively large uncertainty (±1.2%).

Figure 10-3. The sensitivity of ground-level UV flux to a 1 DU change in total column O_3, under clear sky conditions, as a function of solar zenith angle (SZA).

Source: Krotkov et al. (1998).

10.2.1.4   Modeling Surface UV-B Irradiance

1   The WMO/UNEP (2003) stated that, in principle, if the spatial distribution of all UV

2 absorbers and scatterers were fully known, the wavelength and angular distribution of the UV

3 irradiance at the earth's surface could be determined with model calculations. However, the

4 poorly known and complicated distribution of the primary components (clouds, particles, O_3,

5 and surface albedo) makes detailed predictions extremely difficult.

6   Each of the factors described above must be integrated with human behaviors that also

7 influence potential exposure to UV-B radiation in order to calculate the human health risks

8 associated with small changes in total column O_3 density. These human factors and the related

9 health consequences are described in detail below.

1   **10.2.2 Factors Governing Human Exposure to Ultraviolet Radiation**

2   Multiple, complex factors determine the solar flux of UV radiation at the ground level, as

3   discussed in Section 10.2.1. Figure 10-4 illustrates some of these geophysical/atmospheric

4   factors including stratospheric and tropospheric O_3, clouds, aerosols, and Rayleigh scattering.

5   An estimate of the impact of changes in surface-level O_3 on UV-related human health effects

6   requires information about human physical, behavioral, and demographic factors associated with

7   exposure to UV radiation. In this section, the various factors that govern the degree of human

8   exposure to UV radiation are examined.

9
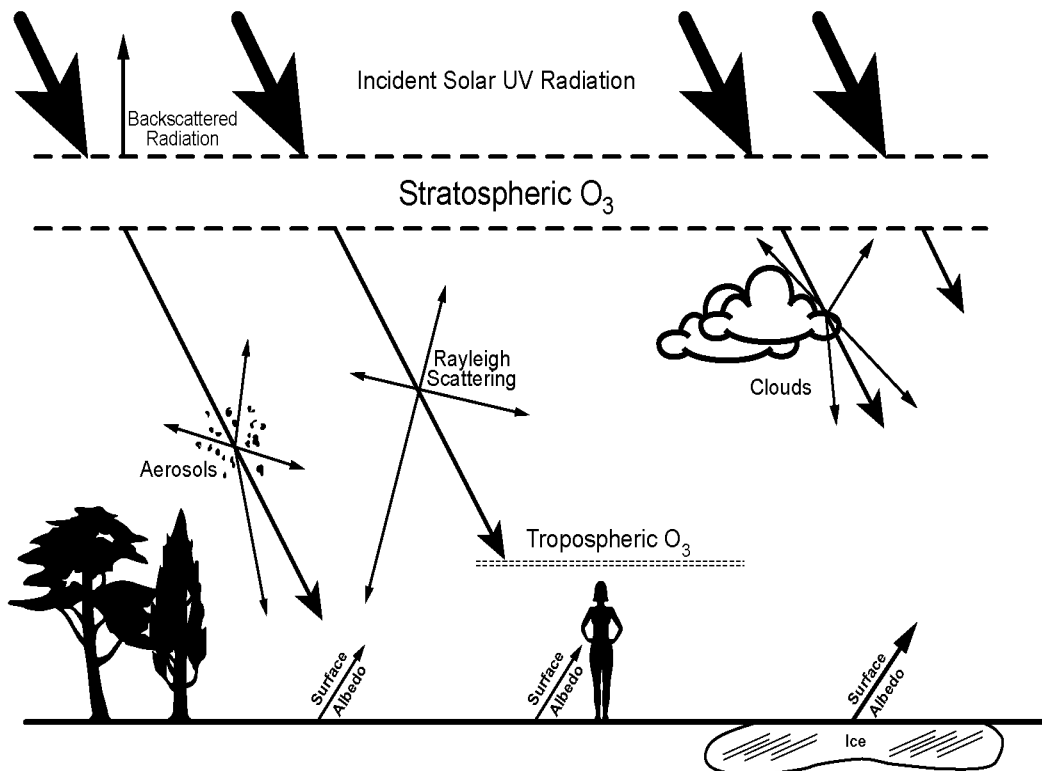
**Figure 10-4. Complexity of factors that determine human exposure to UV radiation.
In addition to the geophysical/atmospheric factors (e.g., stratospheric and
tropospheric O_3, clouds, aerosols, and Rayleigh scattering) that affect the
solar flux of UV radiation at surface level, there are human physical,
behavioral and demographic factors that influence human exposure to
UV radiation.**

10.2.2.1 Outdoor Activities

Exposure to solar UV radiation is related to one predominating factor – time spent in outdoor microenvironments during daylight hours. A large U.S. study was conducted using the EPA National Human Activity Pattern Survey (NHAPS) to assess UV radiation dose in Americans (Godar, 2001; Godar et al., 2001, 2003). The EPA NHAPS recorded the activity profiles of 9,386 Americans (age 0 to 60+ years) over a 24-month period to assess their exposure to various environmental pollutants, plus UV radiation. This study indicated that there was a strong seasonal preference for outdoor activities, with people spending the most time outdoors during the summer, followed by spring, fall, and, lastly, winter (Godar et al., 2001). Because the solar erythemal (i.e., skin reddening) UV radiation dose is also highest during the summer, the estimated UV radiation dose of Americans was more than ten-fold greater in the summer compared to the winter season (Godar et al., 2001). Note that the error associated with estimating UV radiation dose from exposure surveys and one EPA UV-monitoring site located at each quadrant of the U.S. will be high.

Vacationing at the beach during the summer was associated with higher UV radiation exposures (Godar et al., 2001; Thieden et al., 2001). Even after accounting for sunscreen use at the beach, the erythemal UV radiation doses were more than 40% higher during a three-week beach vacation compared to a three-week stay at home (Godar et al., 2001). Danish children and adolescents were found to receive > 50% of their annual UV radiation dose while vacationing at European beaches (Thieden et al., 2004a). The high UV radiation dose received at beaches is due to increased time spent outdoors during daylight hours and increased risk behavior, namely sunbathing. Sunbathing also was associated with increased annual UV radiation dose in the Canadian National Survey on Sun Exposure and Protective Behaviours (Shoveller et al., 1998). Among the 3,449 adults (age 25+ years) who completed the telephone household survey, 21% stated that they spent time actively sunbathing. In a Danish study with 164 participants, all children (age 1 to 12 years) and teenagers (age 13 to 19 years) and 94% of adults (age 20 to 76 years) had days with risk behavior (Thieden et al., 2004b). Teenagers had the highest annual UV radiation doses, as compared to children and adults, a finding likely attributable to their having the highest number of risk-behavior days. Among teenagers, 76% of their UV radiation dose during the measurement period was received on risk-behavior days, as determined using personal electronic UV dosimeters and exposure diaries (Thieden et al., 2004b). In addition to

vacationing and sunbathing, participation in outdoor sports (e.g., basketball, soccer, golfing, swimming, cycling) also significantly increased UV radiation exposure (Moehrle, 2001; Moehrle et al., 2000; Moise et al., 1999; Thieden et al., 2004a,b).

10.2.2.2 Occupation

Of the various factors that affect human exposure to UV radiation, occupation is also important. Approximately 5% of the American workforce perform work in outdoor microenvironments, as determined by the EPA NHAPS (Godar et al., 2001). American indoor workers, on average, spend ~10% of their day outdoors and are exposed to ~30% of the total ground-level UV flux, as measured by the EPA UV-monitoring program, during this time period (Godar et al., 2001). In comparison to indoor or home workers, outdoor workers are exposed to much higher levels of UV radiation (Kimlin et al., 1998a; Thieden et al., 2004a), frequently at levels that are above current exposure limits set by the International Commission on Non-Ionizing Radiation Protection (ICNIRP, 2004). For example, Thieden et al. (2004a) observed that the annual UV radiation dose, estimated using personal electronic UV dosimeters and exposure diaries, ~70% higher for gardeners than indoor workers. The gardeners received the majority (55%) of their UV radiation dose on working days (Thieden et al., 2004a). Another study found that outdoor workers received three to four times the annual UV radiation exposure of indoor workers (Diffey, 1990). At-risk working populations include farmers (Airey et al., 1997; Schenker et al., 2002), fisherman (Rosenthal et al., 1988), landscapers (Rosenthal et al., 1988), building and construction workers (Gies and Wright, 2003), physical education teachers (Vishvakarman et al., 2001), mail delivery personnel (Vishvakarman et al., 2001), and various other workers who spend the majority of their day in outdoor microenvironments during peak UV radiation hours.

10.2.2.3 Age

Age-related differences in UV radiation dose was examined in a U.S. study using the EPA NHAPS (Godar, 2001; Godar et al., 2001). The average UV radiation dose among American children (age < 12 years) was estimated to be similar to that of adults (age 20+ years), but ~20% higher than that of adolescents (age 13 to 19 years) (Godar, 2001). During the summer, higher UV radiation doses were observed in children < 5 years of age and men 40+ years of age (Goder,

1   2001). In a large Canadian survey, 89% of children (age < 12 years) had 30 minutes or more of

2   daily UV exposure, compared to 51% for both adults (age 25+ years) and youth (age 15 to

3   24 years) (Lovato et al., 1998a, 1998b; Shoveller et al., 1998). In an English study (Diffey et al.,

4   1996), UV radiation exposure was estimated in 180 children (age 9 to 10 years) and adolescents

5   (age 14 to 15 years) using personal film badges and exposure records. Once again, children were

6   found to have received higher UV radiation exposure compared to adolescents (Diffey et al.,

7   1996). However, in contrast to the results from the studies discussed above, a Danish study

8   observed that the annual UV radiation dose in teenagers (age 13 to 19 years) was ~20% higher

9   compared to children (age 1 to 12 years) (Thieden et al., 2004b). This increase in UV radiation

10   dose in teenagers was attributed to their increased risk-behavior days. The time profiles of daily

11   UV radiation exposure among grade 8 students was assessed in an Australian study using a

12   routinely operating UV-Biometer and questionnaires (Moise et al., 1999). The results indicated

13   that up to 47% of the daily UV radiation dose fell within the time periods when students were

14   outdoors during school hours, sitting under shaded structures during lunch breaks and

15   participating in routine outdoors or sports activities (Moise et al., 1999).

16   Two studies examined lifetime UV radiation exposure among persons in the U.S. (Godar

17   et al., 2001) and Denmark (Thieden et al., 2004b). Both studies observed that while there are

18   slight differences in UV radiation dose by age, generally people receive fairly consistent UV

19   doses at different age intervals throughout their lives.

20

21   **10.2.2.4 Gender**

22   Studies have indicated that females generally spend less time outdoors and, consequently,

23   have lower UV radiation exposure compared to males (Gies et al., 1998; Godar et al., 2001;

24   Shoveller et al., 1998). The U.S. study by Godar et al. (2001) observed that even though both

25   males and females had somewhat consistent erythemal UV radiation doses throughout their

26   lives, males received consistently higher UV doses compared to females at all age groups.

27   Among all Americans, the lowest exposure to UV radiation was received in females during their

28   child-raising years (age 22 to 40 years) (Godar et al., 2001). The highest exposure was observed

29   in males aged 41 to 59 years in the U.S. study (Godar et al., 2001), while a similar Canadian

30   survey found that younger adult males had the greatest exposures to UV radiation (Shoveller

31   et al., 1998).

1 **10.2.2.5 Geography**

2   In the U.S. study by Godar et al. (2001), erythemal UV radiation doses were examined in

3 persons living in northern and southern regions. Northerners and southerners were found to

4 spend an equal amount of time outdoors; however, the higher solar flux at lower latitudes

5 significantly increased the annual UV radiation dose for southerners (Godar et al., 2001). The

6 annual UV radiation doses in southerners were 25 and 40% higher in females and males,

7 respectively, compared to northerners (Godar et al., 2001). Other studies also have shown that

8 altitude and latitude influence personal exposure to UV radiation (Kimlin et al., 1998b; Rigel

9 et al., 1999).

10

11 **10.2.2.6 Protective Behavior**

12   Protective behaviors such as using sunscreen (e.g., Nole and Johnson, 2004), wearing

13 protective clothing (e.g., Rosenthal et al., 1988; Sarkar, 2004; Wong et al., 1996), and spending

14 time in microenvironments with shaded surfaces (Moise et al., 1999; Parisi et al., 1999) have

15 been shown to reduce exposure to UV radiation. In one study, the use of sunscreen was

16 associated with extended intentional UV radiation exposure (Autier et al., 1999); however, a

17 follow-up study indicated that sunscreen use increased duration of exposures to doses of UV

18 radiation that were below the threshold level for erythema (Autier et al., 2000).

19   In a national study of U.S. youths aged 11 to 18 years, the most prevalent protective

20 behavior was sunscreen use (39.2%) followed by use of a baseball hat (4.5%) (Davis et al.,

21 2002). There were significant differences in the use of sunscreen by age group and gender, with

22 the younger age group (age 11 to 13 years) and girls having greater likelihood (47.4 and 48.4%,

23 respectively) of using sunscreen (Davis et al., 2002). The Canadian National Survey on Sun

24 Exposure and Protective Behaviours observed that less than half of the adults (age 25+ years,

25 n = 3,449) surveyed took adequate protective actions (Shoveller et al., 1998). Once again,

26 children (age < 12 years, n = 1,051) were most protected from exposure to UV radiation, with

27 76% using sunscreen and 36% avoiding the sun, as reported by their parents (Lovato et al.,

28 1998a). However, the protection level was still not adequate, as indicated by the high 45% rate

29 of erythema in children. Among Canadian youth (age 15 to 24 years, n = 574), protective

30 actions from UV radiation exposure included wearing a hat (38%) and seeking shade and

31 avoiding the sun between the peak hours of 11:00 a.m. to 4:00 p.m. (26%) (Lovato et al., 1998b).

1 The lowest prevalence of protective behavior among the youth was likely responsible for the

2 highest proportion of erythema (68%) experienced in this age group. A Danish study observed

3 that both children and teenagers applied sunscreen on more days than adults, but teenagers had

4 the most days with erythema, due to their increased risk behavior (Thieden et al., 2004b).

5 A survey in Switzerland of 1,285 individuals, including children and parents, indicated that

6 sunscreen use was the protective action most commonly used, but only at the beach and not in

7 routine daily exposure (Berret et al., 2002). Protective clothing and avoiding the sun were not

8 highly used among these individuals.

9

10 **10.2.2.7 Summary of Factors that Affect Human Exposures to Ultraviolet Radiation**

11   The factors that potentially influence UV radiation doses were discussed in the previous

12 sections and include choice of leisure activities, occupation, age, gender, geography, and

13 protective behavior. Results from the various studies indicate that specific populations may be at

14 risk for higher exposures to UV radiation. Of particular concern are the following potentially

15 susceptible populations:

16   • Individuals who engage in high-risk behavior, viz., sunbathing;

17   • Individuals who participate in outdoor sports and activities, including professional
       athletes;

18   • Individuals who work in outdoor microenvironments with inadequate shade, e.g.,
       farmers, fishermen, gardeners, landscapers, building and construction workers;

19   • Young children; and

20   • Individuals living in geographic areas with higher solar flux (i.e., lower latitudes
       [e.g., Honolulu, HI] and higher altitudes [e.g., Denver, CO]).

21

22 **10.2.3 Factors Governing Human Health Effects due to Ultraviolet Radiation**

23   Ultraviolet radiation occupies a specific region of the electromagnetic spectrum of

24 wavelengths and can be further subdivided into three parts, UV-A (320 to 400 nm), UV-B

25 (280 to 320 nm), and UV-C (200 to 280 nm). Most of the health risks associated with UV

26 radiation exposure are wavelength dependent. Wavelengths < 180 nm are of little practical

27 biological significance as they are readily absorbed in the air (ICNIRP, 2004). Until 1980,

28 it was generally thought that wavelengths < 315 nm were responsible for the most significant

1 adverse UV radiation health effects; however, recent studies have found that the longer

2 wavelengths in the UV-A range also might produce adverse responses at substantially higher

3 doses (ICNIRP, 2004).

4   Action spectra of a given biological response to UV radiation across its spectral range are

5 used to estimate exposure by weighting individual wavelength intensities according to the

6 associated response. The overall effectiveness of the incident flux at inducing the biological

7 response of interest is computed by means of the relationship

8

$$effective\ irradiance = \int_{\lambda} I_{\lambda}\, E_{\lambda}\, d\lambda \qquad (10\text{-}4)$$

9

10 where I_{λ} and E_{λ} are, respectively, the irradiance and its relative effectiveness at wavelength λ.

11   UV-A and UV-B radiations differ in their abilities to initiate DNA damage, cell signaling

12 pathways, and immune alterations. In this section, the various adverse health effects associated

13 with acute and chronic UV radiation exposure will be discussed.

14

15 **10.2.3.1 Erythema**

16 ***Association Between Ultraviolet Radiation Exposure and Erythema***

17   The most conspicuous and well-recognized acute response to UV radiation is erythema, or

18 the reddening of the skin, which is likely caused by direct damage to DNA by UV-B and UV-A

19 radiation (Matsumura and Ananthaswamy, 2004). Indirect oxidative damage also might occur at

20 longer wavelengths (Matsumura and Ananthaswamy, 2004). Skin type appears to play a large

21 role in the sensitivity to UV radiation-induced erythema. The Fitzpatrick classifications for skin

22 types are: (1) skin type I – individuals with extremely sensitive skin that sunburns easily and

23 severely, and is not likely to tan (e.g., very fair skin, blue eyes, freckles); (2) skin type

24 II – individuals with very sensitive skin that usually sunburns easily and severely, and tans

25 minimally (e.g., fair skin, red or blond hair, blue, hazel or brown eyes); (3) skin type

26 III – individuals with sensitive skin that sunburns moderately and tans slowly (e.g., white skin,

27 dark hair); (4) skin type IV – individuals with moderately sensitive skin that sunburns minimally

28 and usually tans well (e.g., white or light brown skin, dark hair, dark eyes); (5) skin type

29 V – individuals with minimally sensitive skin that rarely sunburns and tans deeply (e.g., brown

1 skin); and (6) skin type VI – individuals with nonsensitive skin that never sunburns and tans

2 profusely (e.g., dark skin). A study by Harrison and Young (2002) found that the perceptible

3 minimal erythemal dose was approximately two-fold greater among individuals with skin type

4 IV compared to skin type I, although there was considerable overlap in the minimal erythemal

5 dose among the four skin types. Waterston et al. (2004) further observed that within an

6 individual, erythemal response differed by body site (e.g., abdomen, chest, front upper arm, back

7 of thigh). These differences were likely attributable to body site-specific variations in melanin

8 pigmentation.

9   Kollias et al. (2001) investigated the change in erythemal response following a previous

10 exposure to UV radiation. Body sites that received a second exposure to UV radiation always

11 showed a reduced erythemal response compared to body sites with a single exposure, especially

12 when the first exposure was at levels greater than the minimal erythemal dose. The suppression

13 of erythema was more pronounced when the second exposure was given 48 hours after the first.

14 However, Kaidbey and Kligman (1981) found that multiple exposures to subthreshold doses of

15 UV radiation at 24-hour intervals were found to lower the minimal erythemal dose. Henrisken

16 et al. (2004) observed that the change in threshold depended on skin type. In 49 healthy

17 volunteers with skin types II, III, and IV, just perceptible erythema 24 hours post-exposure was

18 chosen as the minimal erythemal dose. After four days of repeated UV radiation, the minimal

19 erythemal dose was lowered by 40 to 50% in darker-skinned persons. However, among fair-

20 skinned individuals, there was no change in the erythemal threshold dose with repeated exposure

21 to UV radiation.

22   A reference erythema action spectrum was adopted by the Commission Internationale de

23 l'Eclairage (International Commission on Illumination, CIE) in 1987 (McKinlay and Diffey,

24 1987). The CIE erythema action spectrum indicates that UV-B radiation is orders of magnitude

25 more effective per unit dose than UV-A radiation. However, a follow-up study by Diffey (1994)

26 that compared the observed and predicted minimal erythemal doses found that the erythemal

27 sensitivity of skin at longer UV wavelengths (> 350 nm) was greater than predicted from the CIE

28 reference action spectrum.

29

30

1 *Risk of Erythema from Changes in Tropospheric Ozone Levels*

2   There is no literature on the risk of erythema associated with changes in tropospheric or

3 ground-level O_3 levels. However, one study conducted a risk assessment of the effects of

4 stratospheric O_3 depletion on the risk of erythema (Longstreth et al., 1998). Stratospheric O_3

5 depletion will result in only a slight increase influence rate of UV-A as O_3 only absorbs a very

6 little part of this UV spectrum. However, the ground-level UV-B flux will likely increase as O_3

7 absorbs radiation in that wavelength range with high efficiency.

8   The risk analysis by Longstreth et al. (1998) concluded that erythema will not appreciably

9 increase with depletion of the O_3 layer. This is due to the powerful adaptation of the skin to

10 different levels of UV radiation, as evidenced by its ability to cope with changes in UV radiation

11 by season (van der Leun and de Gruijl, 1993). Gradual exposure to increasing UV radiation

12 from the winter to summer leads to decreased sensitivity of the skin. In midlatitudes, the UV-B

13 radiation in the summer is ten-fold greater than in the winter. In contrast, the steady depletion of

14 the O_3 layer has been estimated to likely lead to an ~20% increase in UV-B in 10 years

15 (Longstreth et al., 1998). Such a comparatively small increase in UV radiation throughout the

16 years, therefore, would not seem likely to significantly increase the risk of erythema. Given that

17 the stratospheric O_3 depletion is estimated as not being likely to affect the risk of erythema, one

18 can conclude that changes in ground-level O_3 (which only constitutes no more than 10% of total

19 atmospheric O_3) is not likely to result in increased risk.

20

21 **10.2.3.2   Skin Cancer**

22   According to the Skin Cancer Foundation, one in six Americans will develop skin cancer

23 during their lifetime (Gloster and Brodland, 1996). The three main forms of skin cancer include

24 basal cell carcinoma and squamous cell carcinoma, which are both nonmelanoma skin cancers,

25 and malignant melanoma. Nonmelanoma skin cancers constitute more than one-third of all

26 cancers in the U.S. and ~90% of all skin cancers, with basal cell carcinoma being approximately

27 four times as common as squamous cell carcinoma (Diepgen and Mahler, 2002; ICNIRP, 2004).

28 The incidence of malignant melanoma (3 to 4% of all cancers) is much lower compared to

29 nonmelanoma skin cancers, but melanoma has great metastatic potential and accounts for the

30 majority of skin cancer deaths (Jemal et al., 2004).

1   Exposure to UV radiation is considered to be a major risk factor for all three forms of skin

2   cancer (Gloster and Brodland,1996; Diepgen and Mahler, 2002; IARC, 1992). Ultraviolet

3   radiation is especially effective in inducing genetic mutations and acts as both a tumor initiator

4   and promoter. Keratinocytes have evolved DNA repair mechanisms that correct the damage

5   induced by UV, but mutations do occur, leading to skin cancers that are appearing with

6   increasing frequency (Hildesheim and Fornace, 2004). The relationship between skin cancer and

7   chronic exposure to UV radiation is further explored below, followed by discussion of the

8   influence of O_3 on the incidence of skin cancer.

9

10   **10.2.3.3   Ultraviolet Radiation Exposure and the Incidence of Nonmelanoma**
11   **Skin Cancers**

12   The incidence of all three types of cancers has been shown to rise with increasing UV

13   radiation concentrations across the U.S. (de Gruijl, 1999); however, the most convincing

14   evidence for a causal relationship exists between UV radiation and squamous cell carcinoma.

15   Squamous cell carcinoma occurs almost exclusively on skin that is regularly exposed to the sun,

16   such as the face, neck, arms, and hands. The incidence is higher among whites in areas of lower

17   latitudes, where solar flux is greater (Kricker et al., 1994). The risk of squamous cell carcinoma

18   was shown to increase with life-long accumulated exposure to UV radiation in one cross-

19   sectional study (Vitasa et al., 1990), but was found to be associated only with the exposure ten

20   years prior to diagnosis in a case-control study (Gallagher et al., 1995a). One of the major

21   concerns with both types of studies is the potential for recall bias in reporting their UV radiation

22   exposure as individuals are already aware of their disease status.

23   Ultraviolet radiation also has been linked to basal cell carcinoma. Basal cell carcinoma is

24   common on the face and neck (80-90%) but rarely occurs on the back of the hands (de Gruijl,

25   1999). While cumulative UV radiation exposure was not associated with risk of basal cell

26   carcinoma (Vitasa et al., 1990), increased risk was observed in individuals with increased

27   recreational UV radiation exposure in adolescence and childhood (age < 19 years) and

28   individuals with a history of severe erythema in childhood (Gallagher et al., 1995b). Once again,

29   consideration must be given to potential recall bias in assessing these results. Thus, there is

30   suggestive evidence that UV radiation also plays a role in the development of basal cell

31   carcinoma, but the etiologic mechanisms for squamous cell carcinoma and basal cell carcinoma

32   likely differ. In an Australian study conducted in a subtropical community, having fair skin,

1   a history of repeated sunburns, and nonmalignant solar skin damage diagnosed by dermatologists

2   were strongly associated with both types of nonmelanoma skin cancer (Green et al., 1996).

3   Outdoor occupation was not associated with nonmelanoma skin cancer, which was likely due to

4   significant self-selection. Individuals with fair or medium complexions and a tendency to

5   sunburn, though they accounted for more than 80% of the community study sample, were

6   systematically underrepresented among outdoor workers (Green et al., 1996). This selection bias

7   may partly explain the lack of consistent quantitative evidence of a causal link between UV

8   radiation and skin cancer in humans.

9   De Gruijl et al. (1993) assessed the action spectrum for nonmelanoma skin cancers using

10   hairless albino mice. Human data are not available to examine the wavelength dependence of

11   the carcinogenicity of UV radiation. After adjusting for species differences, the Skin Cancer

12   Utrecht-Philadelphia action spectrum indicated the highest effectiveness in the UV-B range with

13   a maximum at 293 nm, which dropped to 10^{-4} of this maximum at the UV-A range above

14   340 nm (de Gruijl et al., 1993). The mutations commonly present in the *p53* tumor suppressor

15   gene in individuals with squamous cell carcinoma and basal cell carcinoma are called the

16   "signature" mutations of UV-B radiation (de Gruijl, 2002). UV-B radiation is highly mutagenic

17   due to the fact that DNA is a chromophore for UV-B, but not for UV-A, radiation (Ichihashi

18   et al., 2003). However, other studies have found that UV-A radiation, in addition to UV-B

19   radiation, can induce DNA damage (Persson et al, 2002; Ruenger et al., 2000). DNA damage by

20   UV-A is mediated by reactive oxygen species, thus is indistinguishable from damage caused by

21   other agents that generate reactive oxygen species (de Gruijl, 2002). Epidemiologic evidence of

22   a carcinogenic effect of UV-A was found in a study of psoriasis patients receiving oral psoralen

23   and UV-A radiation treatment (Stern et al., 1998). High-dose exposure to oral psoralen and

24   UV-A radiation was associated with a persistent, dose-related increase in the risk of squamous

25   cell cancer, but with much less effect on the risk of basal cell cancer. Therefore, although UV-B

26   radiation has long been considered the main culprit for nonmelanoma skin cancer, limited

27   evidence suggest that UV-A radiation may also play a role.

28   Susceptible populations for nonmelanoma skin cancers include individuals with reduced

29   capacity for nucleotide excision repair, the primary repair mechanism for UV radiation-induced

30   DNA lesions (Ichihashi et al., 2003). At particular risk are individuals with xeroderma

31   pigmentosum, as they have defective nucleotide excision repair in all tissues (Kraemer, 1997;

1 Sarasin, 1999). Skin type also largely affects susceptibility to skin cancer. Of the six skin

2 phenotypes, the most sensitive individuals are those with skin types I and II, who have a fair

3 complexion, blue or green eyes, and red or blond hair (Diepgen and Mahler, 2002). These

4 individuals tend to sunburn easily, tan poorly, and freckle with sun exposure. A history of

5 repeated sunburns also appears to increase the risk of both cancers, while sunburns during

6 childhood are more associated with increased basal cell carcinoma (Gallagher et al., 1995b;

7 Green et al., 1996).

8

9 ***Ultraviolet Radiation and the Incidence of Cutaneous Malignant Melanoma***

10   From 1973 to 1994, the incidence rate of melanoma increased 120.5% along with an

11 increased mortality rate of 38.9% among whites in the U.S. (Hall et al., 1999). The ICNIRP

12 (2004) states that during the past 40 years or so, each decade has seen a two-fold increase in the

13 incidence of malignant melanoma in white populations, with increased incidence observed more

14 prominently in individuals living in lower latitudes. Cutaneous malignant melanoma has

15 mutifactorial etiology, with environmental, genetic, and host factors (Lens and Dawes, 2004).

16 While the major environmental factor of melanoma has been identified as UV radiation

17 exposure, the risk of melanoma appears to depend on the interaction between the nature of the

18 exposure and skin type (Lens and Dawes, 2004).

19   Fears et al. (2002) examined the association between invasive cutaneous melanoma and

20 UV radiation in non-Hispanic whites using a case-control study design. Lifetime residential

21 history was coupled with mid-range UV-B radiation flux measurements to reduce exposure

22 misclassification and recall bias. A 10% increase in the average annual UV-B flux was

23 significantly associated with a 19% increase in individual odds for melanoma in men and a

24 16% increase in women. Whiteman et al. (2001) conducted a systematic review of studies that

25 examined the association between childhood UV radiation exposure and risk of melanoma.

26 Researchers found that ecological studies assessing ambient sun exposure consistently reported

27 higher risks of melanoma among people who resided in an environment with high UV radiation

28 during their childhood (Whiteman et al., 2001). The lack of consistency among the case-control

29 studies was likely due to the varying methods used to assess UV radiation dose.

30   While the evidence is generally suggestive of a causal relationship between UV radiation

31 and malignant melanoma, possibly conflicting data has been observed. For example, the highest

1 occurrence of malignant melanoma is on men's backs and women's legs, areas that do not have

2 prolonged exposure to the sun (Rivers, 2004). This indicates that malignant melanoma tends to

3 occur in sites of intermittent, intense sun exposure (trunk and legs), rather than in areas of

4 cumulative sun damage (head, neck, and arms), in contrast to nonmelanoma skin cancers

5 (Swetter, 2003).

6   The available data conflict with regard to the relative importance of UV-A versus UV-B in

7 inducing melanomas. UV-A has a much higher flux rate at the earth's surface, as it is not

8 absorbed by O_3 and it is able to penetrate more deeply into the skin surface due to its longer

9 wavelength. However, UV-B, as mentioned earlier, is much more energetic and, therefore, more

10 effective in photochemically altering DNA. The individual roles of UV-B and UV-A in the

11 development of cutaneous malignant melanoma have been examined in several studies.

12 A case-control study of 571 patients and 913 matched controls observed an elevated odds ratio

13 for development of malignant melanoma in individuals who regularly used tanning beds, which

14 typically are UV-A sources (Westerdahl et al., 2000). In a study by Setlow et al. (1993),

15 an action spectrum using the tropical fish *Xiphophorus* indicated that UV-A range wavelengths

16 were especially important in malignant melanoma induction. However, an action spectrum

17 using the opossum *Monodelphis domestica* found that the potency of UV-A for melanoma

18 induction was extremely low compared to that of UV-B (Robinson et al., 2000). A recent study

19 by De Fabo et al. (2004) examined the differences in wavelength effectiveness using a

20 hepatocyte growth factor/scatter factor-transgenic mouse model. The epidermal tissue of these

21 transgenic mice behaves very similarly to the human epidermis in response to UV exposure.

22 Given the absence of a mammalian melanoma action spectrum, the standardized CIE erythema

23 action spectrum was used to deliver identical erythemally effective doses. Only UV-B radiation

24 was found to initiate mammalian cutaneous malignant melanoma. UV-A radiation, even at

25 doses considered physiologically relevant, were ineffective at inducing melanoma (De Fabo

26 et al., 2004). Overall, current evidence suggests that UV-B, and not UV-A, is the primary risk

27 factor for malignant melanoma (ICNIRP, 2004).

28   The populations susceptible for malignant melanoma are similar to those for nonmelanoma

29 skin cancers. Once again, individuals with xeroderma pigmentosum or a reduced capacity of

30 nucleotide excision repair are at increased risk (Tomescu et al., 2001; Wei et al., 2003).

31 Individuals with skin types I and II, or the fair-skin phenotype (blue or green eyes; blond or red

1   hair; skin that freckles, sunburns easily, and does not tan), have increased susceptibility to

2   malignant melanoma (Evans et al., 1988; Swetter, 2003; Veierød et al., 2003). However, the

3   incidence of melanoma was also positively associated with UV radiation in Hispanics and blacks

4   (Hu et al., 2004). Although the incidence of melanoma is much lower in Hispanics and blacks

5   compared to whites, melanomas in these populations are more likely to metastasize and have a

6   poorer prognosis (Black et al., 1987; Bellows et al., 2001). Among children, malignant

7   melanoma appears to have similar epidemiologic characteristics to the adult form of the disease

8   (Whiteman et al., 1997). Individuals with intermittent, intense sun exposure, particularly during

9   childhood, were found to have increased risk of melanoma (Whiteman et al., 2001), in contrast

10 to the association between cumulative exposure and increased risk of squamous cell carcinoma.

11 One study found that a personal history of nonmelanoma skin cancer or precancer, higher

12 socioeconomic status, and increased numbers of nevocytic nevi also were associated with

13 increased incidence of melanoma (Evans et al., 1988).

14

15 ***Effect of Changes in Tropospheric Ozone Levels on Skin Cancer Incidence***

16   The current evidence strongly suggests a causal link between exposure to UV radiation and

17 the incidence of both nonmelanoma and melanoma skin cancer. Genetic factors, including skin

18 phenotype and ability to repair DNA, affect an individual's susceptibility to skin cancer.

19 Quantifying the relationship between UV radiation and skin cancer is complicated by the

20 uncertainties involved in the selection of an action spectrum and appropriate characterization of

21 dose (e.g., peak or cumulative levels of exposure, childhood or lifetime exposures). In addition,

22 there are multiple complexities in attempting to quantify the effect of tropospheric O_3 levels on

23 UV-radiation exposure, as described in Section 9.11.2. There is an absence of published studies

24 that critically examine any increased incidence of skin cancer that may be attributable to

25 decreased tropospheric O_3 exposures (which reflects the significant challenges in determining

26 ground-level O_3-related changes in UV radiation exposure).

27   Several studies have examined the potential effect of stratospheric O_3 depletion on the

28 incidence of skin cancer (de Gruijl, 1995; Longstreth et al., 1995; Madronich and de Gruijl,

29 1993; Slaper et al., 1996). Stratospheric O_3 depletion is likely to increase the ground-level UV-B

30 flux, as O_3 absorbs radiation in that wavelength range with high efficiency. Because UV-B

31 radiation is primarily implicated in the induction of skin cancer, especially among persons with

1 skin phenotypes I and II, there is concern that the depletion of the O_3 layer will result in

2 significantly increased incidence of skin cancers.

3   Estimation of the increased risk in melanoma associated with O_3 depletion cannot be done

4 adequately due to the lack of a mammalian action spectrum for melanoma. In addition, the

5 complexity of the UV-related induction mechanism of melanoma adds an additional layer of

6 uncertainty to the calculations. The excess risk in nonmelanoma skin cancers associated with a

7 decrease in stratospheric O_3 was estimated using the Skin Cancer Utrecht-Philadelphia action

8 spectrum based on hairless albino mice (Longstreth et al., 1995). Quantification of how much

9 more UV radiation would reach ground level with each percentage decrease in O_3 required

10 several assumptions: (1) annual doses are an appropriate measure; (2) personal doses are

11 proportional to ambient doses; and, most notably, (3) each percentage decrease in O_3 is

12 associated with a 1.2% increase in UV radiation. Next, the relationship between UV radiation

13 and nonmelanoma skin cancer incidence was determined. Each percent increase in annual UV

14 radiation dose was estimated to cause a 2.5% increase in squamous cell carcinoma and 1.4%

15 increase in basal cell carcinoma over a human lifetime. Incorporating all these factors,

16 Longstreth et al. (1995) calculated that a sustained 10% decrease in stratospheric O_3

17 concentration would result in 250,000 additional nonmelanoma skin cancer cases per year.

18 Madronich and de Gruijl (1993) noted that the largest percent of O_3-induced nonmelanoma skin

19 cancer increases would be at high latitudes, where baseline incidence of skin cancer is usually

20 small. Assuming a phaseout of primary O_3-depleting substances by 1996, as established by the

21 Copenhagen Amendments in 1992, Slaper et al. (1996) estimated that the number of excess

22 nonmelanoma skin cancers in the U.S. caused by O_3 depletion would exceed 33,000 per year

23 (or ~7 per 100,000) around the year 2050.

24   However, estimating the increase in nonmelanoma skin cancer incidence attributable to the

25 depletion of the stratospheric O_3 layer is marred by uncertainty. The following statement by

26 Madronich and de Gruijl (1994) describes the uncertainty of estimating the effect of

27 stratospheric O_3 depletion on the incidence of skin cancer:

28
29   Extrapolating trends and effects of UV into the future is very hypothetical due to
30   uncertainties that arise from atmospheric chemistry, epidemiology, and related
31   disciplines. The values that we calculated are one plausible measure of the
32   magnitude of the O_3-UV effects….The timescales for atmospheric change and skin-
33   cancer development are still far from certain: O_3 reductions are expected to continue
34   well into next century, and the time between UV exposure and development of skin
35   cancer is essentially unknown. . .

1   Therefore, much caution is necessary when assessing and interpreting the quantitative results of

2   excess nonmelanoma skin cancer incidence due to stratospheric O_3 depletion. Note that although

3   the effect of changes in ground-level or tropospheric O_3 concentrations on skin cancer incidence

4   has not been assessed, it would be expected to be much less compared to the effect from the

5   depletion of the stratospheric O_3 layer, given that tropospheric O_3 makes up $\leq$ 10% of the total

6   atmospheric O_3.

7

8   **10.2.3.4   Ocular Effects of Ultraviolet Radiation Exposure**

9   ***Ultraviolet Radiation Exposure and Risk of Ocular Damage***

10   Ocular damage from UV radiation exposures includes effects on the cornea, lens, iris, and

11   associated epithelial and conjunctival tissues. Absorption of UV radiation differs by

12   wavelength, with short wavelengths (< 300 nm) being almost completely absorbed by the

13   cornea, whereas longer wavelengths are transmitted through the cornea and absorbed by the lens

14   (McCarty and Taylor, 2002). The most common acute ocular effect of environmental UV

15   exposure is photokeratitis, also known as snowblindness, caused by absorption of short

16   wavelength UV radiation by the cornea. The action spectrum indicated that maximum

17   sensitivity of the human eye was found to occur at 270 nm (ICNIRP, 2004; Pitts, 1993). The

18   threshold for photokeratitis in humans varied from 4 to 14 mJ/cm^2 for wavelengths 220 to

19   310 nm. For UV-A radiation, levels exceeding 10 J/cm^2 were necessary for corneal injury.

20   Exposure to longer wavelengths has been shown to cause both transient and permanent

21   opacities of the lens, or cataracts. There is extensive toxicologic and epidemiologic evidence

22   supporting the causal association between UV radiation and cataracts (Hockwin et al., 1999;

23   McCarty and Taylor, 2002). Ultraviolet radiation-induced cataracts are hypothesized to be

24   caused by oxidative stress leading to increased reactive species in the lens, which then causes

25   damage to lens DNA and cross-linking of proteins. Exposure time to low-dose UV radiation was

26   found to strongly influence cataract formation (Ayala et al., 2000). An action spectrum

27   determined using young female rats indicated that the rat lens was most sensitive to 300 nm,

28   correcting for corneal transmittance (Merriam et al., 2000). Oriowo et al. (2001) examined the

29   action spectrum for cataract formation using whole cultured lens from pigs. As pigs lens are

30   similar in shape and size to the human lens, some inferences may be made. Results indicated

31   that the 270 to 315 nm waveband was most effective in producing UV-induced cataracts in vitro.

1   However, the threshold values varied widely within that range, from 0.02 J/cm^2 for 285 nm to

2   0.74 J/cm^2 for 315 nm (Oriowo et al., 2001). At wavelengths > 325 nm, the threshold levels

3   were orders of magnitude larger, with a minimum threshold value of 18.7 J/cm^2. A U.S. study of

4   838 watermen found that UV-B radiation was significantly associated with cortical, but not

5   nuclear, cataract formation (Taylor et al., 1988). No association was observed between cataracts

6   and UV-A radiation in this outdoor-working population.

7

8   ***Risk of Ocular Damage from Changes in Tropospheric Ozone Levels***

9   Cataracts are the most common cause of blindness in the world. McCarty et al. (2000)

10   calculated that ocular UV radiation exposure accounted for 10% of the cortical cataracts in an

11   Australian cohort of 4,744 individuals from both urban and rural areas. A study by Javitt and

12   Taylor (1994-1995) found that the probability of cataract surgery in the U.S. increased by 3% for

13   each 1E decrease in latitude. These results suggest that depletion of the stratospheric O_3 layer

14   may increase UV radiation-induced cataract formation. After assuming a certain wavelength

15   dependency along with some additional assumptions, every 1% decrease in the stratospheric O_3

16   layer was estimated to be associated with a 0.3 to 0.6% increase in cataracts (Longstreth et al.,

17   1995). Longstreth et al. (1995) noted that this estimate has a high degree of uncertainty due to

18   inadequate information on the action spectrum and dose-response relationships. Quantitative

19   estimates have not been possible for photokeratitis, pterygium, or other UV-related ocular effects

20   due to lack of epidemiologic and experimental data. As is the case for all of the other UV-

21   related health outcomes, there is no published information on the potential effects of decreased

22   tropospheric O_3 concentrations on cataract formation due to any changes in surface-level UV

23   flux resulting from decreases in tropospheric O_3.

24

25   **10.2.3.5 Ultraviolet Radiation and Immune System Suppression**

26   Experimental studies have suggested that exposure to UV radiation may suppress local and

27   systemic immune responses to a variety of antigens (Clydesdale et al., 2001; Garssen and van

28   Loveren, 2001; Selgrade et al., 1997). In rodent models, these effects have been shown to

29   worsen the course and outcome of some infectious diseases and cancers (Granstein and Matsui,

30   2004; Norval et al., 1999). Granstein and Matsui (2004) stated that exposure to UV-B radiation

31   caused immunosuppression in mice ultimately by releasing cytokines that prevent antigen-

1 presenting cells from performing their normal functions and causing direct damage to epidermal

2 Langerhans cells. Noonan et al. (2003) investigated UV skin cancer induction in two strains of

3 reciprocal F1 hybrid mice and found that genetically determined differences in susceptibility to

4 UV-induced immunosuppression was a risk factor for skin cancer. At high-UV radiation doses,

5 mice with greater susceptibility to immune suppression had a larger proportion of skin tumors

6 compared to those with low susceptibility (Noonan et al., 2003).

7   While results from animal models are supportive of an association between UV radiation

8 and local and systemic immunosuppression, evidence for UV-induced immunosuppression in

9 humans is limited. There is some evidence that UV radiation has indirect involvement in viral

10 oncogenesis through the human papillomavirus (Pfister, 2003). Additional evidence of

11 UV-related immunosuppression comes from an epidemiologic study of 919 patients with rare

12 autoimmune muscle diseases from 15 cities on four continents with variable UV radiation

13 intensity (Okada et al., 2003). Ultraviolet radiation was strongly associated with the prevalence

14 of dermatomyositis, an autoimmune disease distinguished by the presence of photosensitive

15 pathognomonic rashes (Okada et al., 2003). In patients with the human immunodeficiency virus,

16 UV-B radiation lead to activation of the virus in their skin through the release of cytoplasmic

17 nuclear factor kappa B (Breuher-McHam et al., 2001). In a study by Selgrade et al. (2001),

18 UV-induced immunosuppression was examined in 185 subjects with different skin

19 pigmentations. To assess immune suppression, dinitrochlorobenzene was applied to irradiated

20 buttock skin 72 hours after irradiation. Differences in sensitivity were not related to skin type

21 based on the Fitzpatrick classification or minimal erythemal dose (Selgrade et al, 2001).

22 However, erythemal reactivity, assessed by the steepness of the erythemal dose-response curve,

23 was shown to be significantly associated with UV-induced immunosuppression. Only subjects

24 with steep erythemal responses, which included individuals with skin types I through V, showed

25 a dose-response relationship between UV exposure and immune suppression (Selgrade et al,

26 2001).

27   Most action spectrum investigations have concluded that immunosuppression is caused

28 most effectively by the UV-B waveband (Garssen and van Loveren, 2001). The effects of UV-A

29 on local and systemic immunosuppression have been unclear and inconsistent. There is some

30 evidence that high doses of UV-A is protective of immunosuppression induced by UV-B

31 exposure (Halliday et al., 2004). Given the variety of outcomes of immune suppression and

1 possible mechanisms of effect, little detailed information exists on UV radiation action

2 spectrums and dose-response relationships. The available data are insufficient to develop

3 quantitative risk estimates for UV radiation-induced immunosuppression in humans.

4

5 **10.2.3.6 Protective Effects of Ultraviolet Radiation – Production of Vitamin D**

6 Most humans depend on sun exposure to satisfy their requirements for vitamin D (Holick,

7 2004). UV-B photons are absorbed by 7-dehydrocholesterol in the skin, leading to its

8 transformation to previtamin D_3, which is rapidly converted to vitamin D_3. Vitamin D_3 is

9 metabolized in the liver, then in the kidney to its biologically active form. Vitamin D deficiency

10 is known to cause metabolic bone disease among children and adults, but also may increase the

11 risk of many common chronic diseases, including type I diabetes mellitus and rheumatoid

12 arthritis (Holick, 2004). Vitamin D also is capable of inhibiting the growth of various cancer

13 cells in cell cultures. Thus, vitamin D deficiency may lead to increased incidence of carcinomas

14 in various organs (Studzinski and Moore, 1995).

15 Large geographical gradients in mortality rates for a number of cancers in the U.S. are not

16 explained by dietary or other risk factors; therefore, it has been hypothesized that some

17 carcinomas are due to insufficient UV-B radiation. For example, published literature indicates

18 that solar UV-B radiation, by increasing production of vitamin D, is associated with reduced risk

19 of cancer of the breast (Freedman et al., 2002; Garland et al., 1990; Gorham et al., 1990; Grant,

20 2002a; John et al., 1999), colon (Freedman et al., 2002), ovary (Freedman et al., 2002; Lefkowitz

21 and Garland, 1994), and prostate (Freedman et al., 2002; Hanchette and Schwartz, 1992), as well

22 as non-Hodgkin lymphoma (Hartge et al., 1996). Eight other cancers, including bladder,

23 esophageal, kidney, lung, pancreatic, rectal, stomach, and corpus uteri, have been found to

24 exhibit an inverse correlation between mortality rates and UV-B radiation (Grant, 2002b).

25 Using UV-B data from July 1992 and U.S. cancer mortality rates from 1970 to 1994,

26 premature cancer deaths attributable to reduced UV-B exposure were analyzed in an ecologic

27 study (Grant, 2002b). The annual number of premature deaths from various cancers due to

28 lower UV-B exposures was 21,700 for white Americans; 1,400 for black Americans; and 500 for

29 Asian Americans and other minorities. Uncertainty in the estimation of UV-B exposure limits

30 the confidence for the estimates of excess cancer deaths attributable to reduced exposure.

31 No study has assessed the decreased risk of cancer mortality resulting from increased UV

1 radiation attributable to decreased tropospheric O_3 levels, but the change in risk is expected to be

2 unappreciable.

3   In establishing guidelines on limits of exposure to UV radiation, the ICNIRP agreed that

4 some low-level exposure to UV radiation benefits health (ICNIRP, 2004). However, the adverse

5 health effects necessitated the development of exposure limits for UV radiation. The ICNIRP

6 recognized the challenge in establishing exposure limits that would achieve a realistic balance

7 between beneficial and adverse health effects.

8

9 ## 10.2.4 Summary and Conclusions for O_3 Effects on UV-B Flux

10   Increased solar radiation, especially UV-B radiation, can be detrimental as well as

11 beneficial to the exposed human population. Analogously, both positive and negative effects of

12 UV-B radiation would be expected on plant and animal biota, and on man-made materials (e.g.,

13 Van der Leun et al., 1998; U.S. Environmental Protection Agency, 2002). Other environmental

14 factors include the daily nonlinearity of UV-B flux, nonlinear absorption by O_3, and absorption

15 and scattering of UV-B radiation by both stratospheric and tropospheric processes, including

16 ground-level environmental pollutants such as PM and O_3. Surface flux variability of UV-B is

17 partially dependent upon cloud and tree cover, latitude, time of year, and time of day. Given the

18 magnitude of this natural variability, it would be extremely difficult with current instrumentation

19 and atmospheric models to measure and/or estimate a change in the ground-level UV-B flux and

20 to attribute it to small reductions in troposphericO_3 and/or PM.

21   Of equal or even greater importance are changes in human habits of daily activities,

22 recreation, dress, and skin care. Little is known about the impact of variability in these human

23 factors that can modify individual exposure to UV radiation. Most information about potential

24 UV exposure to the human population relies on demographic information. There is some

25 information available on activity patterns (e.g., EPA NHAPS) and protective behaviors (e.g.,

26 Canadian National Survey on Sun Exposure and Protective Behaviors), but the data are

27 inadequate to accurately assess UV radiation exposure in the population. The uncertainties in

28 characterizing UV radiation exposure limit any UV-related health risk assessment.

29   Another difficulty in examining health risks of UV radiation exposure in the population is

30 due to the lack of detailed information regarding the relevant type (e.g., peak or cumulative) and

31 time period (e.g., childhood or lifetime) of exposure, wavelength dependency of biological

1   responses, and interindividual variability in UV resistance. For example, exposure to solar UV-

2   B radiation appears to be the most important environmental risk factor for basal and squamous

3   cell carcinomas and cutaneous malignant melanoma in fair-skinned individuals. Originally, total

4   cumulative exposure to UV-B was believed to be the most important environmental factor in

5   determining risk for these tumors. New information now suggests that only squamous cell

6   carcinoma risk is related to total accumulated exposure. For basal cell carcinoma and for

7   malignant melanoma, new information suggests that increases in risk are linked to early

8   exposures (before about age 15), particularly those leading to severe sunburns. There is also

9   controversy regarding the effect of UV-A on health outcomes. Though most studies have found

10 that UV-B radiation is highly mutagenic, some evidence indicates that UV-A radiation, at high

11 doses, may also be carcinogenic.

12   Susceptibility to the various UV radiation-induced health effects, generally, appears to be

13 related to skin type. Numerous studies have shown that individuals with Fitzpatrick skin types I

14 and II (i.e., fair-skinned phenotypes) are at increased risk for erythema and skin cancer.

15 In addition, individuals with a reduced ability to repair DNA have increased susceptibility to

16 both nonmelanoma and melanoma skin cancers. For UV-related immunosuppression, however,

17 it appears that erythemal reactivity, rather than skin type, is a marker for susceptibility.

18   There is some evidence that increased UV radiation exposures resulting from depletion of

19 the stratospheric O_3 layer will lead to increases in skin cancer and cataracts. However, the

20 numerous assumptions necessary to calculate these quantitative risk estimates lead to high levels

21 of uncertainty. No study has yet examined the benefits of increased UV radiation exposure due

22 to enhanced production of vitamin D. With the currently available information, the effect of

23 changes in tropospheric O_3 on UV-induced health outcomes cannot be critically assessed.

24

25

26 **10.3   TROPOSPHERIC OZONE AND CLIMATE CHANGE**

27   Water vapor, CO_2, O_3, N_2O, CH_4, CFCs, and other polyatomic gases present in the earth's

28 troposphere, trap infrared radiation emitted by the earth's surface, leading to surface warming.

29 This phenomenon is widely known as the "Greenhouse Effect" (Arrhenius, 1896), and the gases

30 involved are known as "greenhouse gases" (GHGs). The term used for the role a particular

31 atmospheric component, or any other component of the greater climate system, plays in altering

the earth's radiative balance is "forcing." In the past decade, the global atmospheric sciences

and climate communities have made significant progress in determining the specific role O_3

plays in forcing climate.

The Intergovernmental Panel on Climate Change (IPCC) was founded in 1988 by the

World Meteorological Society (WMO) and the United Nations Environmental Program (UNEP)

to support the work of the Conference of Parties (COP) to the United Nations Framework

Convention on Climate Change (UNFCCC). Drawing from the global climate and atmospheric

sciences community for its authors and reviewers, the IPCC produces reports containing

thorough assessments of the available, peer-reviewed science regarding the physical climate

system, past and present climate, and evidence of human-induced climate change. This section

will summarize the reviews of the available information on the forcing properties of tropospheric

O_3 provided by IPCC Third Assessment Report (IPCC, 2001a) will be given, and will describe

some of the more recent developments on the subject.

The projected effects of global climate change will be briefly explained to provide the

context within which O_3 serves as a regional, and possibly global, anthropogenic pollutant.

The concept of climate forcing is also explained, along with the factors that influence the extent

of climate forcing by ozone. The section concludes with a summary of the various estimates that

have been placed on the globally-averaged forcing due to ozone.

10.3.1   The Projected Impacts of Global Climate Change

The study of the atmospheric processes involved in global climate change and its potential

consequences for human health and global ecosystems is an area of active research. The IPCC

Third Assessment Report (TAR) is the most thorough evaluation of current scientific

understanding of climate change available at this time. In addition to the first and second IPCC

assessments in 1990 and 1995, along with other IPCC reports, earlier assessments included those

conducted by the United Nations Environment Program (UNEP, 1986), the World

Meteorological Organization (WHO, 1988), the U.S. Environmental Protection Agency (1987),

and others (e.g., Patz et al., 2000a,b). The reader is referred to these documents for a complete

discussion of climate change science. An abbreviated list of the IPCC conclusions, to date, and a

short discussion of the potential impacts of climate change on human health and welfare is

1   provided here to serve as the context for the discussion of the role of the increasing tropospheric

2   O_3 concentration in climate change.

3   According to various historic and modern measurement records, atmospheric GHG

4   concentrations have increased dramatically in the past century, and have been attributed to

5   human activities. The IPCC TAR describes the scientific theory and evidence tying increases

6   in GHGs to human activities (IPCC 2001a).

7   An increasing body of geophysical observations shows that the earth is warming and that

8   other climate changes are underway. These observations include the global surface temperature

9   record assembled since the year 1860, the satellite temperature record begun in 1979, recorded

10   changes in snow and ice cover since the 1950s, sea level measurements taken throughout the

11   20th century, and sea surface temperature observations recorded since the 1950s. Other

12   evidence includes a marked increase over the past 100 years in the frequency, intensity, and

13   persistence of the zonal atmospheric circulation shifts known as the El Nino-Southern

14   Oscillation (ENSO). ENSO events occur when the tropical Pacific Ocean has accumulated a

15   large, localized mass of warm water that interrupts cold surface currents along South America,

16   altering precipitation and temperature patterns in the tropics, subtropics and the midlatitudes.

17   IPCC (1998, 2001a) reports also describe the results of general circulation model (GCM)

18   studies that predict that human activities will alter the climate system in a manner that will likely

19   lead to marked global and regional changes in temperature, precipitation and other climate

20   properties. These changes are expected to increase the global mean sea level, as well as increase

21   the number of extreme weather events including floods and droughts, increased wind speeds and

22   precipitation intensity of tropical cyclones, and changes in soil moisture. These predicted

23   changes can be expected to directly impact human health, ecosystems, and global economic

24   sectors, e.g., hydrology and water resources, food and fiber production (IPCC, 1998, 2001b).

25   Table 10-1 summarizes these projected impacts.

26   Wide variations in the course and net impacts of climate change in different geographic

27   areas are expected. In general, the projected changes will result in additional stresses on natural

28   ecosystems and human societal systems already impacted by increasing resource demands,

29   unsustainable resource management practices, and pollution. Some of the predicted changes

30   include alterations in ecological balances; in the availability of adequate food, water, clean air;

Table 10-1. Examples of Impacts Resulting From Projected Changes in Extreme Climate Events

Projected changes during the 21st Century in Extreme Climate Phenomena and their Likelihood[a]	Representative Examples of Projected Impacts[b] *(all high confidence of occurrence in some areas[c])*
Simple Extremes	
Higher maximum temperatures; more hot days and heat waves[d] over nearly all land areas (*very likely*[a])	• Increased incidence of death and serious illness in older age groups and urban poor • Increased heat stress in livestock and wildlife • Shift in tourist destinations • Increased risk of damage to a number of crops • Increased electric cooling demand and reduced energy supply reliability
Higher (increasing) minimum temperatures; fewer cold days, frost days, and cold waves[d] over nearly all land areas (*very likely*[a])	• Decreased cold-related human morbidity and mortality • Decreased risk of damage to a number of crops, and increased risk to others • Extended range and activity of some pest and disease vectors • Reduced heating energy demand
More intense precipitation events (*very likely*[a] over many years)	• Increased flood, landslide, avalanche, and mudslide damage • Increased soil erosion • Increased flood runoff could increase recharge of some floodplain aquifers • Increased pressure on government and private flood insurance systems and disaster relief
Complex Extremes	
Increased summer drying over most midlatitude continental interiors and associated risk of drought (*likely*[a])	• Decreased crop yields • Increased damage to building foundations caused by ground shrinkage • Decreased water resource quantity and quality • Increased risk of forest fire
Increase in tropical cyclone peak wind intensities, mean and peak precipitation intensities (*likely*[a] over some areas)[e]	• Increased risk to human life, risk of infections, disease epidemics, and many other risks • Increased coastal erosion and damage to coastal buildings and infrastructure • Increased damage to coastal ecosystems such as coral reefs and mangroves
Intensified droughts and floods associated with El Niño events in many different regions (*likely*[a]) (see also under droughts and intense precipitation events)	• Decreased agricultural and rangeland productivity in drought- and flood-prone regions • Decreased hydro-power potential in drought-prone regions
Increased Asian summer monsoon precipitation variability (*likely*[a])	• Increased flood and drought magnitude and damages in temperate and tropical Asia

Table 10-1 (cont'd). Examples of Impacts Resulting From Projected Changes in Extreme Climate Events

Projected changes during the 21st Century in Extreme Climate Phenomena and their Likelihood[a]	Representative Examples of Projected Impacts[b] *(all high confidence of occurrence in some areas[c])*
Increased intensity of midlatitude storms (little agreement between current models)[d]	• Increased risks to human life and health • Increased property and infrastructure losses • Increased damage to coastal ecosystems

[a]Likelihood refers to judgmental estimates of confidence used by TAR WGI: *very likely* (90-99% chance); *likely* (66-90% chance). Unless otherwise stated, information on climate phenomena is taken from the Summary for Policymakers, TAR WGI. TAR WGI = Third Assessment Report of Working Group 1 (IPCC, 2001a).
[b]These impacts can be lessened by appropriate response measures.
[c]High confidence refers to probabilities between 67 and 95%.
[d]Information from TAR WGI, Technical Summary.
[e]Changes in regional distribution of tropical cyclones are possible but have not been established.

Source: IPCC (2001b).

1 and in human health and safety. Poorer nations can be expected to suffer the most, given their

2 limited adaptive capabilities.

3 Although many regions are predicted to experience severe, possibly irreversible, adverse

4 effects due to climate change, beneficial changes may also take place. For example, certain

5 regions may benefit from warmer temperatures or increased CO_2 fertilization, e.g., U.S. West

6 Coast coniferous forests, some Western rangelands. Specific benefits may include reduced

7 energy costs for heating, reduced road salting and snow-clearance costs, longer open-water

8 seasons in northern channels and ports, and improved agricultural opportunities in the northern

9 latitudes, as well as the Western interior and coast. For further details about the projected effects

10 of climate change on a U.S.-regional scale, the reader is also referred to several regionally-

11 focused reports (MARAT, 2000; Yarnal et al., 2000; NERAG, 2001; GLRAG, 2000), as well as

12 a report on potential impacts upon human health due to climate change (Bernard et al.,

13 2001).The IPCC report, "The Regional Impacts of Climate Change," (IPCC, 1998) describes the

14 projected effects of human-induced climate change on the different regions of the globe,

15 including Africa, the Arctic and Antarctic, the Middle East and arid Asia, Australasia, Europe,

16 Latin America, North America, the small island nations, temperate Asia, and tropical Asia.

17 While climate models are successful in simulating present annual mean global climate and the

1   seasonal cycles on continental scales, they have been less successful on regional scales. Clouds

2   and humidity, essential factors in defining local and regional (sub-grid scale) climate, are

3   significantly uncertain (IPCC, 2001a). Due to modeling uncertainties, both in reproducing

4   regional climate and in predicting the future economic activity that will govern future GHG

5   emissions, the projected impacts discussed above are also uncertain.

6   Findings from the United States Global Change Research Program (USGCRP) (NAST,

7   2000) report and related reports illustrate the considerable uncertainties and difficulties in

8   projecting likely climate change impacts on regional or local scales. The USGCRP findings also

9   reflect the mixed nature of projected potential climate change impacts (i.e., combinations of

10   mostly deleterious, but other possibly beneficial, effects) for U.S. regions and their variation

11   across different regions. Difficulties in projecting region-specific climate change impacts are

12   complicated by the need to evaluate the potential effects of regional- or local-scale changes in

13   key air pollutants not only on global-scale temperature trends, but also regional- or local-scale

14   temperature and precipitation patterns.

15

10.3.2   Solar Energy Transformation and the Components of the Earth's Climate System

18   Mass, in any form, has the capacity to interact with solar and terrestrial radiation, but the

19   manner in which mass interacts with radiation is governed by its particular physical form and/or

20   molecular properties. The most interesting example of radiative properties as a function of

21   physical form is water. In its gas phase, water is the most important GHG present in the climate

22   system, due to its ability to absorb long-wave terrestrial radiation. Conversely, in its frozen form

23   as snow or sea ice, the most important role for water in the climate system is scattering

24   ultraviolet and visible solar radiation back to space, i.e., decreasing the earth's net solar radiation

25   receipts by increasing the earth's reflective properties (albedo). In its liquid aerosol form as

26   clouds, water scatters radiation. In its bulk liquid form as ocean water, it absorbs terrestrial

27   radiation, and represents the earth's most important reservoir of heat energy due to its mass.

28   Molecular properties become important in comparing the radiative absorptive capacity of

29   different gases. In the case of gases, the atomic composition and molecular structure determine

30   the wavelengths of light that a gas molecule can absorb. Ozone and O_2, provide one example of

31   the importance of molecular structure in determining absorption capacity. These molecules are

composed solely of atomic oxygen atoms, but their bond structures are distinct. Ozone, because

of its three-atom, bent molecular structure, has the capacity to absorb terrestrial (infrared)

wavelengths, making it a GHG. Tropospheric O_3, like stratospheric O_3, also has the capacity to

absorb ultraviolet radiation of 320 nm and shorter, increasing the energy-absorbing capacity of

the troposphere. Molecular oxygen, O2, due to its structure, is limited to absorbing very short-

wave ultraviolet light – and does so at altitudes too high to influence the climate system

significantly.

Each component of the climate system plays a role in absorbing, transforming, storing,

dispersing, or scattering solar radiation. Weather is a consequence of the transformation and

dispersion of terrestrial radiation. The term, "weather," refers to the condition of the earth's

atmosphere at a given time and place. It is defined by the air temperature, air pressure, humidity,

clouds, precipitation, visibility, and wind speed. The "climate" for a given place on the earth's

surface is a long-term average of these elements of weather, accounting for daily and seasonal

weather events. The frequency of extreme weather events is used to distinguish among climates

that have similar averages (Ahrens, 1994).

The earth's capacity for retaining heat is increased by the transformation of its oxygen, for

example, into O_3 by way of air pollution chemistry. Climate components, including GHGs, land,

oceans, sea ice, land ice and snow, atmospheric particles, vegetation, clouds, etc., all contribute

to the earth's heat capacity with respect to solar energy. Changes in the properties (or mass) of

these components will "force" the climate system in one direction or the other, i.e., warmer

versus cooler. Climate forcing is further described, below.

10.3.3 The Composition of the Atmosphere and the Earth's Radiative Equilibrium

The Greenhouse Effect is the term given to the decreased rate of reemission of absorbed

solar energy due to the heat-retaining properties of the Earth's atmosphere. According to simple

radiative transfer theory, at thermal equilibrium, the earth's temperature should be near -15 °C.

This is the temperature of a theoretical "black body" that is receiving and then reemitting

342.5 Wm^{-2}, i.e., the globally-averaged amount of full-spectrum solar energy absorbed, and then

reemitted by the earth as infrared terrestrial radiation, per square meter. In fact, satellite

observations well above the atmosphere indicate that the earth's average *planetary* temperature

1   is remarkably close to its theoretical black body value at − 18 °C, a temperature at which liquid

2   water ordinarily does not exist.

3   At its *surface*, however, the earth's average temperature is +15 °C. The +33 °C

4   temperature differential between the Earth's planetary and surface temperatures is due to the

5   presence of infrared radiation-absorbing components in the atmosphere, such as water vapor,

6   CO_2, CH_4, several other trace gases, and some types of particles and clouds.

7   The atmosphere, when cloud free, is largely transparent in the solar wavelength range.

8   A small fraction of this radiation is absorbed and reemitted as black body radiation by dark

9   atmospheric particles (IPCC, 2001a). The majority of clouds and particles offset, in part, the

10 greenhouse effect by increasing the earth's albedo and, therefore, decreasing the overall amount

11 of solar radiation absorbed by the earth system.

12   Ozone, SO_2 and NO_2 also absorb ultraviolet and near ultraviolet wavelengths, in addition to

13 infrared wavelengths. Once absorbed by a gas molecule, the energy introduced by a photon may

14 induce a photochemical reaction with the residual energy thermally exciting (heating) the

15 products of the reaction. Alternatively, the energy introduced into the molecule by the photon

16 may be dispersed amongst neighboring molecules via intermolecular collisions, or reemitted in

17 part as a lower energy (IR) photon.

18   Radiation from the sun or the earth's surface that is absorbed by gases and particles is

19 reemitted isotropically, i.e., it is equally likely to be emitted in all directions. Therefore, to a

20 first approximation, half of the radiation trapped by the earth's atmosphere is reflected back to

21 its surface. A portion of this radiation is transformed into the heat energy that drives the

22 atmospheric processes that form the basis of weather and climate. Radiation which is not

23 absorbed by gases and aerosols reaches the earth's surface where it is scattered (reflected) or

24 absorbed, depending on the surface albedo.

25   Successful modeling of the earth's climate and, therefore, the assessment of the extent of

26 human-induced climate change and development of appropriate policy depends on the quality of

27 available information on the relative efficiencies, amounts, and spatial and temporal distributions

28 of the various radiatively active components of the atmosphere that absorb and/or reflect solar

29 and terrestrial radiation, along with all the other non-atmospheric components of the earth

30 system.

31

10.3.3.1 Forcing of the Earth's Radiative Balance

As mentioned earlier, the commonly used measure of the relative influence of a given component of the climate system on the earth's radiative balance is its radiative forcing (IPCC, 2001a; Houghton et al., 1990). Radiative forcing, in Wm^{-2}, is a quantity that was developed by the climate modeling community as a first order-only means of estimating relative effects of individual anthropogenic and natural processes on the energy balance within the climate system. Discussions within the climate community are underway regarding a metric to replace forcing as the standard measure of climate impact – one that will account for more of the factors that determine the effectiveness of a given atmospheric component in this capacity. However, forcing remains the current standard (National Research Council, 2005).

When the effect of a particular component of the climate system is to reduce the amount of solar energy absorbed, usually by increasing the earth's albedo, this component is said to provide a "negative" forcing, measured in Wm^{-2}. The convention assigns a positive value to the forcing induced by climate system components that enhance the greenhouse effect, or otherwise act to increase the heat absorbing capacity of the earth system. Purely reflective atmospheric aerosol, clouds, white roof tops, snow-covered land surfaces, and dense sea ice provide a negative forcing, while highly absorbing dark-colored atmospheric aerosols, GHGs, and increases, due to the melting of sea ice sheets, in dark ocean surfaces positively force the climate system.

Global and regional climate are roughly defined by the balance between the large number of positive and negative forcings induced by the many different components of the earth system. However, the earth system responds to these forcings in a complex way, due to feedback mechanisms that are theorized but very difficult to resolve at the quantitative level.

A simple example would be the positive feedback associated with melting sea ice. As sea ice melts with increasing surface temperatures, the dark ocean surface is revealed which is more efficient at absorbing infrared radiation, further increasing the rate of warming. The role of feedbacks in determining the sensitivity of climate to changes in radiative forcing is described in detail in the IPCC TAR (IPCC, 2001a). In the absence of complete, quantitative, information about climate feedbacks the use of radiative forcing values for the many components of the climate system remains the primary method for estimating their relative importance in climate change.

1   The IPCC has reported estimated values for forcing by individual radiatively active gases,

2 and particle-phase components of the atmosphere, derived primarily through expert judgment

3 incorporating the results of peer-reviewed modeling studies. The forcing estimates, shown in

4 Figure 10-5, are global averages attributed to known greenhouse gases, including O_3; particles;

5 anthropogenic cirrus clouds; land-use change; and natural solar flux variations. Uncertainty

6 ranges are assigned to reflect the range of modeled values reported in those studies. The current

7 estimate of forcing due to long-lived, well-mixed, greenhouse gases accumulated in the

8 atmosphere from the preindustrial era (ca., 1750) through the year 2000 is $+ 2.4$ $Wm^{-2} \pm 10\%$

9 (IPCC, 2001a). An indication of the level of confidence in each of these estimates is given along

10 the bottom of this figure, again reflecting the expert judgment of the IPCC.

11

12
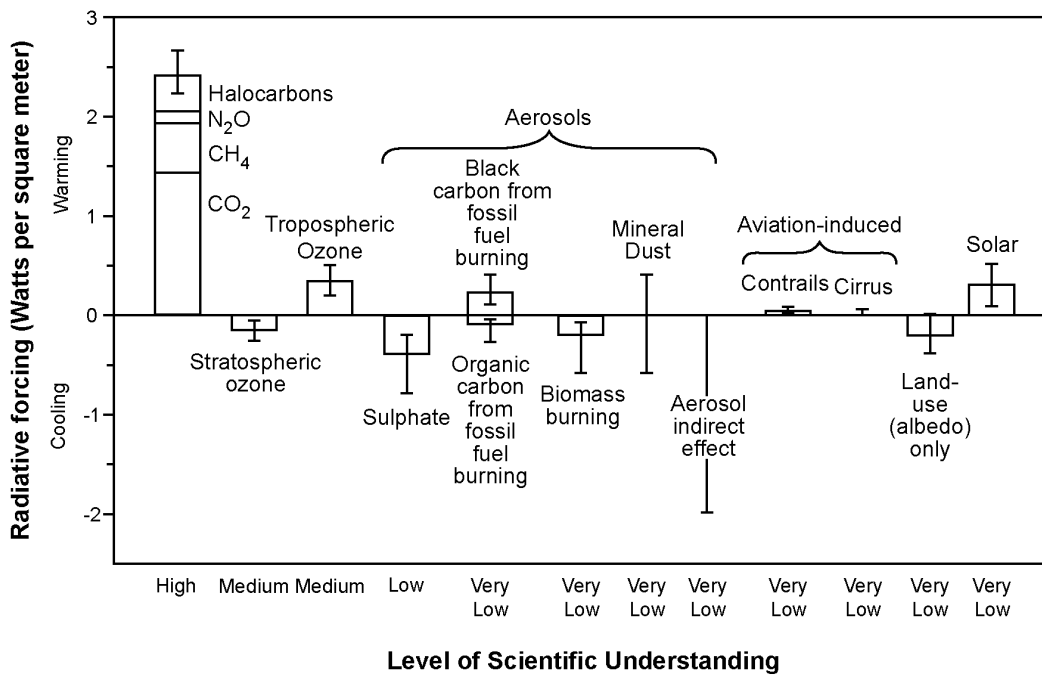
Figure 10-5. **Estimated global mean radiative forcing exerted by gas and various particle
phase species for the year 2000, relative to 1750.**

Source: IPCC (2001a).

1   The IPCC reported a global average forcing value of 0.35 ± 0.15 Wm^{-2} for tropospheric O$_3$,

2   based on model calculations constrained by climatological observations. The considerations and

3   studies used to estimate this value will be outlined below.

4

10.3.4   Factors Affecting the Magnitude of Climate Forcing by Ozone

6   Tropospheric O$_3$ is estimated to have provided the third largest increase in direct forcing

7   since pre-industrial times. It may also play a role in indirect forcing through its participation in

8   the oxidative removal of other radiatively active trace gases, such as CH$_4$ and the HCFCs. Given

9   its relatively short atmospheric lifetime, the distribution of tropospheric O$_3$ is highly variable in

10   geographic extent and time. The direct and indirect forcing it imposes on the climate system,

11   therefore, depends upon its geospatial and temporal distribution, but also depends upon the

12   albedo of the underlying surface. These several variables introduce substantial uncertainty into

13   tropospheric ozone forcing estimates.

14

10.3.4.1   Global versus Regional Atmospheric Ozone Concentrations

16   Ozone reacts photochemically at time-scales generally shorter than those for large-scale

17   mixing processes in the atmosphere. Concentrated O$_3$ plumes evolve downwind of strong

18   sources of its precursor pollutants: reactive nitrogen, CO, and non-methane hydrocarbons

19   (NMHCs). The most important of these sources are midlatitude industrialized areas and tropical

20   biomass burning. When viewed from above the atmosphere by satellite-borne spectrometers, O$_3$

21   enhancements appear as relatively localized air masses or regional-scale plumes, usually

22   originating from industrialized areas or areas in which active biomass burning is underway. The

23   IPCC (2001a) describes the efforts of several research teams who have analyzed data supplied by

24   the satellite-borne Total Ozone Mapping Spectrometer (TOMS) and other remote-sensing

25   instruments to map the global distribution of tropospheric O$_3$ and to attempt to identify processes

26   that influence the global tropospheric O$_3$ budget (IPCC, 2001a). More recently, coincident

27   observations of total O$_3$ by TOMS and the Solar Backscattered UV (SBUV) instrument were

28   used by Fishman et al. (2003) to construct well-resolved spatial and temporal maps of the

29   regional distribution of tropospheric O$_3$. Their results were consistent with those reported by

30   others, but with higher regional-scale resolution. They reported large O$_3$ enhancements in the

31   southern tropics in austral Spring, and in the northern temperate latitudes in the summer. The

1 regional nature of high O_3 concentrations was clearly visible in northeastern India, the eastern

2 United States, eastern China, and west and southern Africa, each coincident with high population

3 densities. Fishman et al. (2003) noted, as have the other groups cited above, significant

4 interannual variability in the concentrations observed over these regions. *In situ* measurements

5 of tropospheric O_3 concentrations range from 10 ppb over remote oceans, to 100 ppb in both the

6 upper troposphere and in plumes downwind from polluted metropolitan regions (IPPC, 2001a).

7 Ground-level concentrations in urban areas are often higher than 100 ppb. In the southern

8 hemisphere, one of the largest sources of O_3 precursors is biomass burning. Biomass burning

9 elevates O_3 on large spatial scales, particularly in the tropical Atlantic west of the coast of Africa

10 and in Indonesia.

11   Current estimates place the global burden of tropospheric O_3 at a highly uncertain

12 370 Tg, equivalent to an average column density of 34 Dobson Units (1 DU = 2.687×10^{16}

13 molecules/cm^{-2}) or a mean concentration of 50 ppb (IPCC, 2001a). Accounting for differences

14 in levels of industrialization between the hemispheres, the average column burden in the

15 Northern Hemisphere is estimated to be 36 DU, with the Southern Hemisphere estimated to

16 average 32 DU. Due to its rapid photochemistry, individual surface measurements of

17 tropospheric O_3 cannot capture large scale concentrations, nor will it represent the concentration

18 at higher altitudes. Dense surface and vertical measurements (ozonesondes) would be required

19 to supplement available output from remote sensing instruments to provide the complete set of

20 observations needed to derive a credible global O_3 budget. Such a measurement program

21 appears, at present, to be impractical.

22   Little historical data exists that might be used to estimate the global ozone burden prior to

23 industrialization. Although a few late 19th century measurements suggest that O_3 has more than

24 doubled in Europe during the 20th century. The insufficient data record on pre-industrial

25 tropospheric O_3 distributions introduces a major uncertainty in the estimation of the ozone-

26 induced forcing (IPCC, 2001a).

27

28 **10.3.4.2   Global Versus Regional Atmospheric Ozone Trends**

29   For the northern hemisphere, weekly continuous data since 1970 are available from only

30 nine stations in the latitude range 36˚N to 59˚N (IPCC, 2001a). Available tropospheric O_3

31 measurements do not reveal a clear trend in concentration, while trends in the stratosphere are

1 more readily identified. Different trends are seen at different locations for different periods,

2 consistent with regional changes in pollutant emission, especially NO_x. Logan et al. (1999)

3 analyzed the composite record of mid-tropospheric ozone abundance from the nine station

4 network. A plot of data is shown in Figure 10-6. While no clear trend appeared for 1980

5 through 1996, the second half of this record (about 57 ppb) is clearly greater than the first half

6 (about 53 ppb). The trend may be consistent with changes in regional NOx emission rates due to

7 pollution reduction efforts in developed countries and increasing emissions in rapidly growing

8 economies in Asia.

9

10
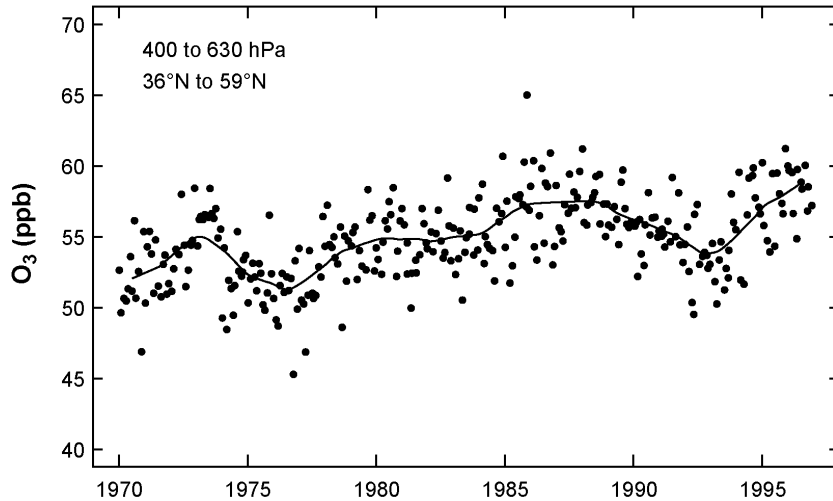
Figure 10-6. Mid-tropospheric O_3 abundance (ppb) in northern midlatitudes (36 °N-59 °N) for the years 1970 to 1996. Observations between 630 and 400 hPa are averaged from nine ozone sonde stations (four in North America, three in Europe, two in Japan), following the data analysis of Logan et al. (1999). Values are derived from the residuals of the trend fit with the trend added back to allow for discontinuities in the instruments. Monthly data (points) are shown with a smoothed 12-month-running mean (line).

Source: IPCC (2001).

1   It must be noted that the measurements shown in Figure 10-6 are for surface

2 concentrations, only. Many fewer locations have measured changes in the concentrations of O_3

3 as a function of altitude. Fewer still are locations that have collected and maintained data

1   records prior to 1970. The absence of historical data on the vertical distribution of O_3 adds to the

2   difficulty in estimating historical atmospheric burdens and trends in O_3-related climate forcing.

3   The IPCC (2001a) surveyed the results of published chemistry transport model (CTM)

4   modelling studies, listed in Table 10-2, that estimated the global average increase since the

5   pre-industrial era in total column O_3. Model estimates ranged from +7 to +12 DU. On the basis

6   of these estimates, available measurements and other analyses, the IPCC estimated that total

7   column O_3 has increased by 9 DU, with a 67% confidence range of +6 to +13 DU. In some of

8   the modelling studies, emissions scenarios were used that predict a further increase in column

9   O_3 due to growing emissions of O_3 precursors.

10

11

**Table 10-2. CTM Studies Assessed by the IPCC for its Estimate of the Change in
Global, Total Column O_3 Since the Pre-Industrial Era**

Estimated Change in Column Ozone in DU	Model Used	Authors (Publication Date)
7.9	GFDL	Haywood et al. (1998)
8.9	MOZART-1	Hauglustaine et al. (1998)
8.4	NCAR/2D	Kiehl et al. (1999)
9.5	GFDL-scaled	Levy et al. (1997)
12	Harvard/GISS	Mickley et al. (1999)
7.2	ECHAM4,	Roelofs et al. (1997)
8.7	UKMO,	Stevenson et al. (2000)
9.6	UIO	Berntsen et al. (1999)
8	MOGUNTIA	VanDorland et al. (1997)

Source: IPCC (2001).

1   Chapters 2 and 3 of this document provide an expanded discussion of the issues associated

2   with determining the global tropospheric O_3 background.

3

1 ### 10.3.4.3 The Sensitivity of Ozone-related Forcing Surface to Albedo

2   The characteristics of the surface underlying an O_3 enhancement play a large role in the O_3

3 forcing effect. Highly reflective, warm surfaces, such as light-colored deserts, scatter solar

4 ultraviolet (UV) radiation; absorb, and then emit infrared terrestrial radiation. Both forms of

5 radiation can be trapped, transformed, and/or reemitted back to the surface by tropospheric O_3.

6 Highly reflective, cold, surfaces will scatter more radiation while emitting less terrestrial infrared

7 radiation. Dark, warm, surfaces such as tropical ocean hot spots predominantly emit terrestrial

8 radiation. Ozone will trap heat at differing efficiencies as a consequence of the amount and type

9 of radiation reflected or emitted from the surface underneath it. Studies by two groups,

10 Hauglustaine et al. (1998) and Mickley et al. (1999), have shown that industrial pollution that

11 has been transported to the Arctic induces a high, regional O_3-related forcing due to the highly

12 reflective underlying ice and snow surface.

13

14 ## 10.3.5 Estimated Forcing by Tropospheric Ozone

15 ### 10.3.5.1 Direct Climate Forcing Due to Ozone

16   The inhomogeneous distribution of O_3 within the troposphere coupled with the large

17 uncertainty in the global O_3 budget significantly complicates the matter of estimating the global

18 average direct forcing due to O_3. The IPCC TAR (2001a) lists the results of several modeling

19 studies that estimated the annual change in the relative forcing by O_3 since pre-industrial times.

20 It was noted that the differences amongst the estimates were most likely due to differences in

21 predicted O_3 chemistry, including the emissions inventories used, and the chemical process and

22 transport mechanisms incorporated into the models, rather than by factors relating to radiative

23 transfer. The IPCC intercomparison of the models and their results indicated that the

24 uncertainties in estimated forcings due to O_3 have reduced since the IPPC Second Assessment

25 Report (1996)

26   The O_3-related forcings estimated by studies considered by the IPCC (2001a) are listed in

27 Table 10-3. Ten of the listed estimates are based on global chemistry/transport models

28 calculations. One study was constrained by a climatology derived from observations. Given the

29 differences in calculated total column O_3 amongst the models, a normalized forcing (Wm^{-2} per

30 Dobson Unit of tropospheric O_3 change) is listed in addition to the absolute forcing (Wm^{-2})

Table 10-3. Tropospheric O$_3$ Change (ΔO$_3$) in Dobson Units (DU) Since Pre-industrial Times, and the Accompanying net (SW plus LW) Radiative Forcings (Wm^{-2}), After Accounting for Stratospheric Temperature Adjustment (using the Fixed Dynamical Heating method). Estimates are Taken From the Published Literature. Normalized Forcings (norm.) Refer to Radiative Forcing per O$_3$ Change (Wm^{-2} per DU)

Estimated Global Average Forcing Due to Tropospheric Ozone

Reference		Clear sky conditions		Total sky conditions	
	ΔO$_3$	Net	Net (norm.)	Net	Net (norm.)
Berntsen et al. (1997) – [Reading model]	7.600	0.310	0.041	0.280	0.037
Stevenson et al. (1998)	8.700	0.391	0.045	0.289	0.033
Berntsen et al. (1997) – [Oslo model]	7.600	0.390	0.051	0.310	0.041
Haywood et al. (1998a)	7.900	0.380	0.048	0.310	0.039
Kiehl et al. (1999)	8.400	0.379	0.045	0.320	0.038
Berntsen et al. (2000)	9.600	0.428	0.045	0.342	0.036
Brasseur et al. (1998)	—	—	—	0.370	—
van Dorland et al. (1997)	8.070	0.443	0.055	0.380	0.047
Roelofs et al. (1997)	7.200	0.397	0.055	0.404	0.056
Lelieveld and Dentener (2000)	—	—	—	0.420	—
Hauglustaine et al. (1998)	8.940	0.511	0.057	0.426	0.048
Mean	8.224	0.403	0.049	0.343	0.042

Source: IPCC (2001).

1   estimated by each model. Both clear sky (cloud-free) and total sky (including clouds) forcing

2   estimates are listed.

3   The largest O$_3$-related forcings coincide with strongest sources of tropospheric ozone, the

4   models predict that occur in the northern midlatitude regions (40° to 50° N), reaching as much as

5   1 Wm^{-2} in the summer and in the tropics, related to biomass burning. In general, the estimates

6   are comparable in magnitude and show similarity in geographic distribution. For total sky

7   conditions, the range in globally and annual averaged tropospheric O$_3$ forcing from all of these

8   models is from 0.28 to 0.43 Wm^{-2}, while the normalized forcing is 0.033 to 0.056 Wm^{-2} per DU.

9   As expected, they are opposite in sign to the forcing estimated for sulphate aerosols, which

1   scatter radiation. The range in normalized forcings emphasizes the differences in assumptions

2   used by the different models. The tropospheric O_3 forcing constrained by the observational

3   climatology is 0.32 Wm^{-2} for globally averaged, total sky conditions. As shown in Figure 10-1,

4   the IPCC (2001) concluded that 0.35 ± 0.15 Wm^{-2} represents the most likely value for annually

5   and globally-averaged forcing by tropospheric O_3.

6

7   **10.3.5.2   Indirect Forcing Due to Ozone**

8   Ozone has an indirect climate forcing effect due to its role in the oxidative removal of

9   other reactive GHGs, including CH_4, hydrofluorocarbons (HFCs) and other reactive

10   non-methane hydrocarbons (NMHCs). The primary actor in this effect is a second generation

11   product of the photolysis of O_3, the hydroxyl radical (OH). Hydroxyl radical is produced by

12   way of a pair of reactions that start with the photodissociation of O_3 by solar UV.

13

14

15                         $$O_3 + h\nu \rightarrow O(^1D) + O_2 \qquad\qquad (10\text{-}5)$$

16

17                         $$O(^1D) + H_2O \rightarrow OH + OH \qquad\qquad (10\text{-}6)$$

18

19   Reactions with OH are the primary removal mechanism for CH_4 and NMHCs as well as the

20   pollutants NO_x and CO. Methane and CO have especially high abundances in the global

21   atmosphere. OH is estimated to react with these two gases within one second of its formation.

22   In addition to CH_4, NO_x, CO and the NMHCs, OH concentrations are controlled by local

23   concentrations of H_2O (humidity) and the intensity of solar UV. Different atmospheric

24   concentrations of the required precursors suggest that pre-industrial OH concentrations are likely

25   to have been different from present-day concentrations, but there is no consensus on the

26   magnitude of this difference. Observations of global atmospheric concentrations of chloroform

27   (CH_3CCl_3), a well-mixed tropospheric species that reacts with OH, have been used to estimate

28   OH abundances. Independent studies have shown overlapping trends for the period 1978 to

29   1994, but none outside the given uncertainty ranges (0.5 ± 0.6%/yr) (Prinn et al., 1995; Krol

30   et al., 1998). The IPCC (2001) reported a range of +5% to -20% for predicted changes in global

31   OH abundances.

1   Given the difficulty in estimating global OH abundances in the past, present and future,

2   estimates of indirect forcing due to O_3 have been difficult to obtain and are highly uncertain.

3   Attempts have been made to account for OH feedbacks in estimating the global warming

4   potential of CH_4.

5

10.3.5.3 Predictions for Future Climate Forcing by Anthropogenic Ozone

7   The rate of increase in surface O_3 in Europe and North America, since 1980, appears to be

8   slowing, likely due to control measures intended to improve urban air quality. Not surprisingly,

9   CTM modeling attempts to predict future precursor emissions and resulting O_3 abundances

10   indicate that the largest future O_3-related forcings will be related to population growth and

11   economic development in Asia (van Dorland et al., 1997; Brasseur et al., l998). The results of

12   these modeling studies suggested that a higher globally averaged total radiative forcing due to O_3

13   from pre-industrial times to 2050 of 0.66 Wm^{-2} and 0.63 Wm^{-2}. Chalita et al. (1996) predicted a

14   globally averaged radiative forcing from pre-industrial times to 2050 of 0.43 Wm^{-2}. Stevenson

15   et al. (l998) predicted an O_3-related forcing of 0.48 Wm^{-2} in 2100. All of these predictions must

16   be viewed with much caution given the considerable uncertainties associated with such

17   estimates.

18

10.3.6 Conclusion

20   The general consensus within the atmospheric sciences community, as represented by the

21   United Nations Intergovernmental Panel on Climate Change (IPCC), is that human activities

22   have a discernable effect on the earth's climate. However, quantifying the extent of human-

23   induced forcing on climate requires detailed information about human-induced change on the

24   components of the earth system that govern climate. Troposphere ozone is a well-known GHG,

25   but information regarding its historical trends in concentration, its current and future

26   atmospheric burden, and other critical details needed for estimating its direct and indirect

27   forcing effects on the climate system are highly uncertain.

28   The IPCC has estimated that the globally averaged forcing due to O_3 is approximately

29   0.35 ± 0.15 Wm^{-2}. The role of O_3 in climate is likely to be much more pronounced adjacent to

30   the sources of its chemical precursors, consistent with satellite observations of high regional

31   scale column densities near large urban areas and large-scale biomass burning activity.

1   Modeling studies evaluated by the IPCC have estimated that regional scale forcing due to O_3 can

2   approach 1 Wm^{-2}, or two-thirds the forcing estimated for CO_2. However, more reliable

3   estimates of the overall importance of forcing due to tropospheric O_3 await further advances in

4   monitoring and chemical transport modeling.

5

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11. EFFECT OF OZONE ON MAN-MADE MATERIALS

Ozone and other photochemical oxidants react with many of the economically important man-made materials, decreasing their useful life and aesthetic appearance. Some of the materials known to be damaged by ozone include elastomers, fibers and dyes, and paints. This section will provide a brief discussion on the effects of ozone on man-made materials including the damage mechanisms and, where possible, concentration-response relationships. Since only limited information has been published on the effects of ozone on materials, this section will provide a summary of information presented in the previous ozone criteria document (U.S. Environmental Protection Agency, 1996) and a more detailed discussion of studies published since publication of the previous ozone criteria document. The reader is referred to the previous ozone criteria document for a more detailed discussion of the earlier studies.

11.11.1 Mechanisms of Ozone Damage and Exposure-Response

Elastomer Cracking

The elastomeric compounds, natural rubber and synthetic polymers and copolymers of butadiene, isoprene, and styrene, are particularly susceptible to even low levels of ozone. Elastomeric compounds are long chain unsaturated organic molecules. Ozone damages these compounds by breaking the molecular chain at the carbon-carbon double bond; a chain of three oxygen atoms is added directly across the double bond, forming a five-membered ring structure (Mueller and Stickney, 1970). The change in structure promotes the characteristic cracking of stressed/stretched rubber called "weathering." A 5% tensile strain will produce cracks on the surface of the rubber that increase in number with increased stress/stretching. The rate of crack growth is dependent on the degree of stress, the type of rubber compound, ozone concentration, time of exposure, ozone velocity, and temperature (Bradley and Haagen-Smit, 1951; Lake and Mente, 1992) (Gent and McGrath, 1965). Once cracked, there is further ozone penetration and in additional cracking and eventually mechanical weakening or stress relaxation (U.S. Environmental Protection Agency, 1996). Razumovskii et al. (1988) demonstrated the effect of ozone on stress relaxation of polyisoprene vulcanizates. A decrease in stress (stress relaxation) was caused by ozone-induced cracks in exposed elastomers resulting in irreversible changes in the elastomer dimensions and decreased tensile strength. A description of findings of earlier

1 studies appear in the 1996 Air Quality Criteria Document for ozone (U.S. Environmental

2 Protection Agency, 1996).

3   To counteract the effect of ozone on elastomers, antiozonants and wax have been added to

4 the elastomeric formulations during processing. An antiozonant is an additive used to protect a

5 polymer against the effects of ozone-induced degradation and, hence, used mainly in diene

6 rubbers. Antiozonant protection works either by providing a physical barrier to ozone

7 penetration forming a thin surface film of an ozone-resisting wax or by chemically reacting with

8 ozone or polymer ozonolysis products, as do aromatic diamines such as p-phenylene diamine

9 derivatives. The antiozonant diffuses to the surface of the elastomeric material where it reacts

10 with ozone faster than ozone reacts to break the molecular chain and the carbon-carbon double

11 bond, or the antiozonant diffuses to the surface of the material but is not reactive with ozone and

12 serves as a protective coating against ozone attack. The antiozonant may also serve to scavenge

13 ozone while also providing protective film against ozone attack (Andries et al., 1979; Lattimer

14 et al., 1984).

15   Most of the studies on ozone effects on elastomers were designed to evaluate the

16 effectiveness of antiozonants in counteracting the rubber cracking produced by ozone exposure.

17 Consequently, many of the studies were conducted using ozone concentrations higher than those

18 typically found in the ambient air. Natural rubber strips exposed to high concentrations of ozone

19 (20,000 ppm) under stressed conditions cracked almost instantaneously and were broken within

20 1 sec. When the ozone concentration was lowered (0.02 to 0.46 ppm), the time to required to

21 produce cracks in the exposed rubber material was increased (Bradley and Haagen-Smit, 1951).

22 Lake and Mente (1992) studied the effect of temperature on ozone-induced elastomer cracking

23 and antiozonant protection on natural rubber, epoxidised natural rubber, and two acrylonitrile-

24 butadiene copolymers under constant strain. Temperatures ranged from -20˚ C to +70˚ C. The

25 elastomers were exposed to 0.05 to 1,000 ppm ozone for 16 h. Ozone cracking decreased at

26 lower ambient temperatures, however, diffusing of both chemical and wax antiozonants also

27 were slowed at the lower temperatures. Cracking was slightly increased at the higher

28 temperatures but the antiozonants offered more protection.

29   Serrano et al. (1993) evaluated the appropriateness of using ozone-induced elastomer

30 cracking to estimated the ambient ozone concentrations. Two vulcanized natural rubber

31 compounds were exposed for 24 h to varying ozone concentrations under stressed conditions.

1 Ozone concentrations were 60, 80, 90, 100, and 120 ppb for durations of 2, 4, or 6 h. The 24 h

2 average ozone concentrations ranged from 31 to 57.5 ppb. There was a clear relationship

3 between the 24-h average ozone concentration and the distribution of crack length frequencies

4 on the rubber surface. Table 11-1 gives the average 24-h ozone concentration and lengths for

5 two vulcanized natural rubber strips.

6

7

Table 11-1. Average 24-h Ozone Concentrations Producing the Highest Frequency of Cracks of a Certain Length in the Middle and Central Zones of the Rubber Test Strips

Crack Length (mm)	1% Antiozonant 4010NA #		0.5% Antiozonant 4010NA	
	Middle Zones	Central Zones	Middle Zones	Central Zones
0.05 - 0.10	37.5	37.5	40.0	42.5
0.10 - 0.15	45.0	48.0	48.0	53.0
0.15 - 0.20	48.0	$\geq$ 57.5	$\geq$ 57.5	$\geq$ 57.5
0.20 - 0.40	$\geq$ 57.5	$\geq$ 57.5	$\geq$ 57.5	$\geq$ 57.5

Ozone concentrations given in ppb.

Adapted from Serrano et al. (1993).

1 ## 11.11.2   Textiles and Fabrics

2   Ozone can damage textiles and fabrics by methods similar to those associated with

3 elastomers. Generally, synthetic fibers are less affected by ozone than natural fibers, however,

4 ozone contribution to the degradation of textiles and fabrics is not considered significant (U.S.

5 Environmental Protection Agency, 1996). A study reported by Bogaty et al. (1952) showed that

6 ozone effects moistened cloth more than dry cloth. Scoured cotton duck cloth and commercially

7 bleached cotton print cloth were exposed to 20 to 60 ppb for 1,200 h (50 days). The rate of

8 deterioration was measured by the changes in cuprammonium fluidity values and the fabric

9 breaking strength. At the end of the 1,200-h exposure, there was a 20% loss in breaking

10 strength. Table 11-2 list the changes in cuprammonium fluidity values for both fabrics.

**Table 11-2. Cuprammonium Fluidity of Moist Cotton Cloth Exposed
to 20 to 60 ppb Ozone**

	Duration of Exposure (h)	Cuprammonium Fluidity (rhes)
Duck Cloth	0	2.6
	200	2.8
	680	4.0
	960	6.8
	1200	9.5
Bleached Print Cloth	0	8.2
	200	8.7
	510	9.4
	650	12.0
	865	12.7
	1500	16.5

Adapted from Bogaty et al. (1952).

11.11.3 Dyes, Pigments, and Inks

Ozone fading of textile dyes is diffusion-controlled; the rate of fading is controlled by the diffusion of the dye to the fiber surface. Many textile dyes react with ozone; however, the rate and severity of the ozone attack is influenced by the chemical nature of the textile fiber and the manner in which the dye is applied. Ozone molecules break the aromatic ring portion of the dye molecule, oxidizing the dye (U.S. Environmental Protection Agency, 1996). In case of aromatic azo dyes, ozone attacks the aromatic rings and electron rich nitrogen atoms (Matsui et al., 1988). Grosjean et al. (1987; 1988a,b) proposed a mechanism of reaction of ozone and indigo, thioindigo, and dibromoindigo, alazarin, and curcumin dyes under dark conditions. Ozone attaches to the dye molecule at the unsaturated carbon = carbon bond. An ozone adduct is formed (1,2,3-trioxolane), followed by scission of the carbon–carbon bond and the subsequent formation of the corresponding Criegee biradical. A similar mechanism was proposed for the reaction of ozone with triphenylmethane colorant Basic Violet 14. Ozone attacked Basic Violet 14 at the carbon=carbon unsaturated bond and at the carbon‒nitrogen unsaturated bond under dark conditions. Other members of the group of triphenylmethane colorants with unsaturated carbon–carbon bonds also are expected to be subject to ozone fading. Tripheylmethane colorants that are expected to be ozone-fugitive include the amino-substituted cationic dyes

1 (Malachite Green, Brilliant Green, Crystal Violet, Pararosaniline Chloride, Methyl Green, and

2 others) (Grosjean et al., 1989).

3 An indication that ozone caused textile dye fading was first reported by Salvin and Walker

4 (1955). The researchers found that the fading was primarily the result of the destruction of the

5 blue dye molecule. Drapes made of acetate, Arnel, and Dacron and dyed with anthraquinone

6 blue dye exhibited a decrease in shade that was not accompanied by the characteristic reddening

7 caused by NO_x. Figures 11-1and 11-2 demonstrate the effect of ozone exposure on nylon 6 yarn

8 colored with several blue dyes. Nylon samples inside the home were located on a wall away

9 from sunlight. Outside nylon samples were placed on a covered patio or under the eaves of the

10 house to minimize exposure to sunlight and rain. Ozone concentrations ranged from 2 to 5 ppb

11 outside and 0 to 2 ppb inside. The percent change in dye color was determined monthly by

12 extraction and analysis of the remaining dye or by instrumental measurement of the color change

13 (Haylock and Rush, 1978).

14

15 **11.11.4 Artists' Pigments**

16 Several artists' pigments are sensitive to fading and oxidation by ozone when exposed to

17 concentrations found in urban areas (Shaver et al., 1983; Drisko et al., 1985; Whitmore et al.,

18 1987; Whitmore and Cass, 1988; Grosjean et al., 1993). The organic pigments that are ozone

19 fugitive include alizarin red pigments containing lakes of the polycyclic aromatic compound

20 1,2-dihydroxyanthraquinone, blue-violet pigments containing substituted triphenylmethane

21 lakes, indigo, and yellow coloring agents containing polyfunctional, polyunsaturated compounds

22 such as curcumin (Grosjean et al., 1987). Because of the potential of ozone to damage works of

23 art, recommended limits on ozone concentrations in museums, libraries, and archives are

24 relatively low, ranging from 0.013 to 0.01 ppm.

25 Experimental studies demonstrate a concentration × time (C × T) relationship between

26 ozone concentration and exposure time and pigment fading. Cass et al. (1991) summarized

27 some of the earlier research on the effects of ozone on artists' pigments. In studies evaluating

28 the effect of ozone on organic and inorganic watercolors and traditional organic pigments only

29 the traditional organic pigments showed measurable fading from ozone exposure. Of the

30 inorganic pigments tested, only the arsenic sulfides showed ozone-related changes. The

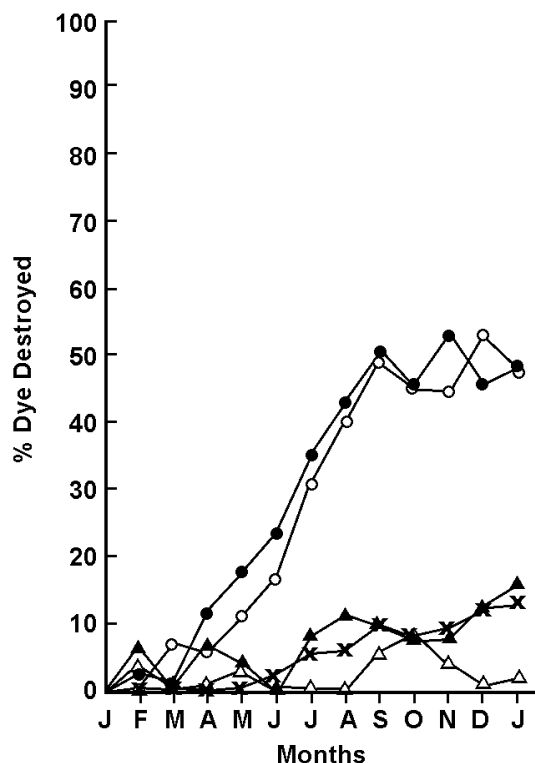pigments were exposed to 0.3 to 0.4 ppm ozone for 3 mo in the absence of light, at 22 °C and

Figure 11-1. **In-service fading of nylon 6 yarn inside house. ● = C.I. Disperse Blue 3; O = C.I. Basic Blue 22; ▲ = C.I. Acid Blue 27; x = C.I. Disperse Blue 56; △ = C.I. Acid Blue 232.**

Source: Haylock and Rush (1978).

1 50% RH. The authors equated this exposure to a C × T of 6 to 8 years inside a Los Angeles

2 museum with air conditioning but without a pollutant removal system.

3   Whitmore and Cass (1988) studied the effect of ozone on traditional Japanese colorants.

4 Most of these compounds are insoluble metal salts that are stable in light and air. Suspensions or

5 solutions of the colorants were airbrushed on hot-pressed watercolor paper or silk cloths.

6 A sample of Japanese woodblock print also was included in the analysis. Samples were exposed

7 to 0.4 ppm ozone at 22 °C, 50% relative humidity, in the absence of light for 12 wk. Changes in

8 reflectance spectra were used to evaluate the effect of ozone exposure on colorant fading.

9 Among the colorants tested on paper, curmin, indigo, madder lake, and lac lake were the most

10 sensitive to ozone exposure. Gamboge was relatively insensitive to ozone.
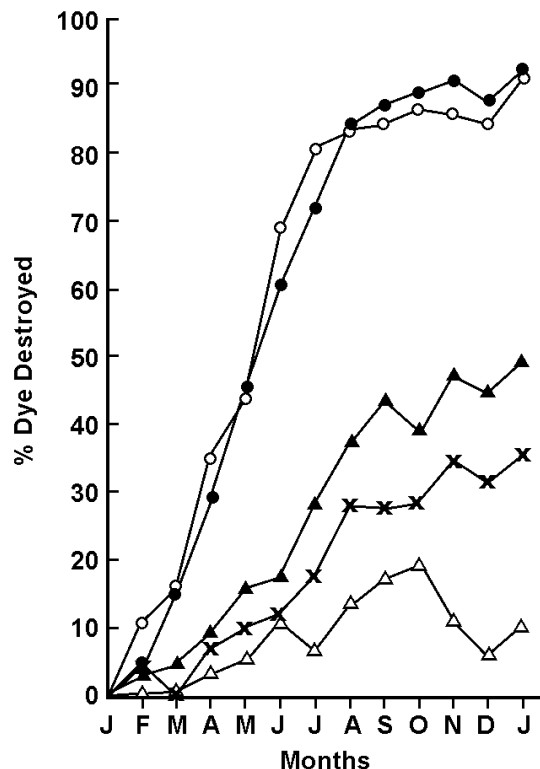
**Figure 11-2.   In-service fading of nylon 6 yarn outside house. ● = C.I. Disperse Blue 3;
O = C.I. Basic Blue 22; ▲ = C.I. Acid Blue 27; x = C.I. Disperse Blue 56;
△ = C.I. Acid Blue 232.**

Source: Haylock and Rush (1978).

1   The blue and green areas of the sample from the woodblock print was very reactive due to the

2   indigo dye ozone sensitivity. The other colorants, red, yellow, and purple, showed very little

3   sensitivity to ozone. The textiles dyes that reacted with ozone were indigo, alone or in

4   combination with several yellow dyes.

5   Ye et al. (2000) reported the rate of ozone fading of traditional Chinese plant dyes. Twelve

6   different colorants were applied to watercolor paper and silk and exposed to 0.4 ppm ozone at

7   25° C, at 50% RH, in the absence of light for 22 wks. Dye fading was greater when the colorant

8   was applied to the watercolor paper compared to the silk cloth due to the darker initial depth of

9   the shade, the greater saturation of the colorant throughout the cloth. Tumeric, gromwell, and

10   violet on paper was particularly reactive. Tangerine peel was moderately reactive and sappan

11   wood, dalbergia wood, Chinese gall, indigo, and Chinese yellow cork tree were slightly reactive

1   to ozone. Black tea was not reactive to ozone. The colorants on silk samples showing color

2   changes were gromwell, sappan wood, gardenia, tummeric, and violet. Figures 11-3 and 11-4

3   demonstrate the color change of the various colorants on watercolor paper and silk.

4   Artists' pigments also have exhibited fading when exposed to a mixture of photochemical

5   oxidants. Grosjean et al. (1993) exposed 35 artists' pigments to a mixture of photochemical

6   oxidants consisting of ozone, nitrogen dioxide (NO_2), and peroxyacetyl nitrate (PAN) for

7   12 wks. Weekly average photochemical concentrations were 200 ppb for ozone, 56 ± 12 to

8   99 ± 24 for NO_2, and 11 ± 3 to 18 ± 2 for PAN. All exposures were carried out at room

9   temperature in the absence of light. To determine the effect of humidity on pigment fading, the

10   relative humidity was increased from 46% after 8 weeks of exposure to 83% for a 2 week period

11   and then returned to 46% for the remainder of the exposure.

12   Table 11-3 lists the artists' pigment and degree of fading. Eleven of the pigments

13   exhibited negligible color change, 12 had small color changes, 3 had modest color changes, and

14   9 exhibited substantial color changes. Fading of Disperse Blue 3 and Reactive Blue 2 were

15   likely the result of NO_2 exposure, the fading of triphenylmethanes is consistent with exposure to

16   nitric acid formed under high humidity conditions. Fading of the indigos was dominated by

17   ozone exposure and curcumin was faded by all of the photochemicals studied. Increasing the

18   relative humidity resulted in a substantial color change for all of the pigments, with the

19   exception of curcumin and indigo.

20

21   11.11.5 Surface Coatings

22   Ozone will act to erode some surface coatings (paints, varnishes, and lacquers). However,

23   many of the available studies on ozone degradation of surface coatings do not separate the

24   effects of ozone from other pollutants or environmental factors such as weather, humidity, and

25   temperature. Campbell et al. (1974) attempted to demonstrate an ozone related effect on oil

26   house paint, acrylic latex coating, alkyd industrial maintenance coating, urea alkyd coil coating,

27   and nitrocellulose/acrylic automotive paint. Painted test panels were exposed to 100 and

28   1,000 ppb ozone in a xenon arc accelerated weathering chamber for up to 1,000 h. Using weight

29   loss as a measure of ozone-induced erosion the researchers concluded that all of the paints tested

30   suffered degradation in the presence of ozone and that the automotive finish suffered the most
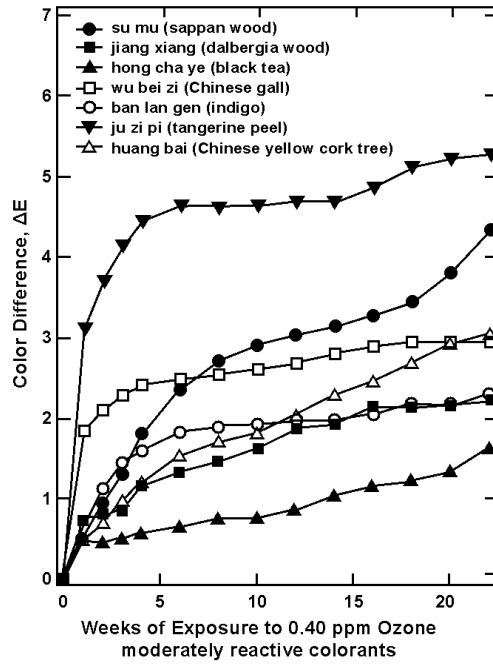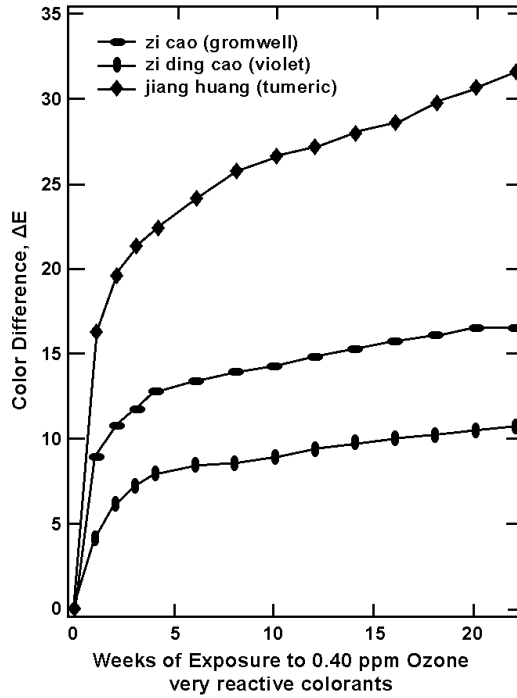
Figure 11-3. Observed color changes for natural colorant-on-paper systems during exposure to 0.40 ppm ozone at 25 °C ± 1 °C, 50% RH, in the absence of light.
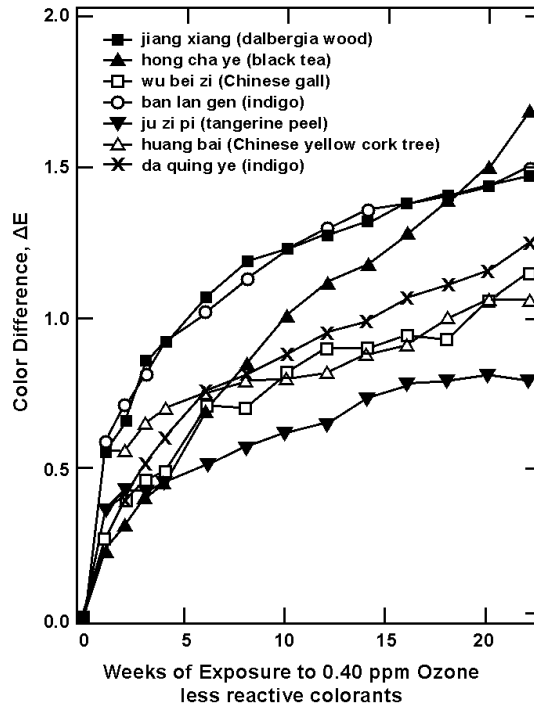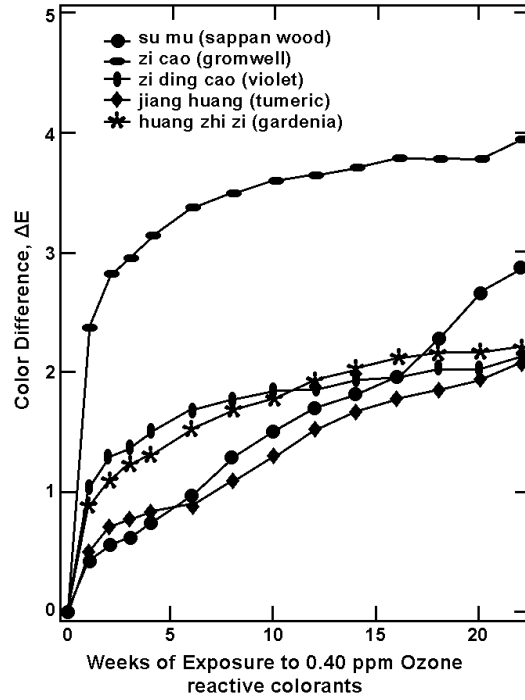
Source: Ye et al. (2000).

Figure 11-4. Observed color changes for natural colorant-on-site during exposure to 0.40 ppm ozone at 25 °C ± 1 °C, 50% RH, in the absence of light.

Source: Ye et al. (2000).

**Table 11-3. Color Change After 12 Weeks of Exposure to a
Mixture of Photochemical Oxidants**

Colorant[*]	Color Change (ΔE units)[†]	Chemical Functionality or Chemical Composition
Acid Red 37 (17045)[‡]	11.7 ± 0.5	Aminophenyl-substituted azo dye, sulfonate salt
Acid Yellow 65 [‡]	1.8 ± 0.5	Nitro- and phenyl-substituted azo dye, sulfonate salt
Alizarin Carmine	1.8 ± 0.2	Alizarin lake
Alizarin Crimson (Pigment Red 83)	1.4 ± 0.2	Alizarin lake
Aurora Yellow (77199)	0.5 ± 0.1	Cadmium sulfide
Basic Fuschin (42510)[‡]	33.4 ± 3.0	Amino-substituted triphenylmethane
Brilliant Green (42040)[‡]	20.6 ± 2.1	Amino-substituted triphenylmethane
Brown Madder	1.7 ± 0.1	Alizarin lake
Cadmium Yellow (77199)	0.4 ± 0.1	Cadmium sulfide
Carmine	1.8 ± 0.2	Lake of cochineal (substituted anthraquinone)
Chrome Yellow (77600)[‡]	1.7 ± 1.2	Lead chromate
Copper phthalocyamne (Pigment Blue 15)	1.0 ± 0.1	Copper phthalocyanine
Crimson Lake	3.5 ± 0.3	Alizarin lake
Curcumin (Natural Yellow 3)	15.2 ± 2.6	1,7 bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione
Disperse Blue 3	10.8 ± 0.1	Amino-substituted anthraquinone
French Ultramarine Blue	0.8 ± 0.3	
Gamboge (Natural Yellow 24)	0.4 ± 0.1	Gambogic acid
Hooker's Green Light	1.5 ± 0.4	Chlorinated copper phthalocyanine plus ferrous beta naphthol derivative
Indigo (a formulation)	1.1 ± 0.1	Alizarin lake plus lampblack plus copper plthalocyanine
Indigo carmine [‡]	14.0 ± 1.9	5,5-indigo disulfonic acid, sodium salt
Indigo (73000)[‡]	64.1 ± 4.5	
Mauve	3.6 ± 0.5	Lake of triphenyl methane (basic fuschin) plus copper phthalyocyanine
New Gamboge	0.9 ± 0.1	Arylamide yellow (CI 11680) plus toluidine red

Table 11-3 (cont'd). Color Change After 12 Weeks of Exposure to a Mixture of Photochemical Oxidants

Colorant[*]	Color Change (ΔE units)[†]	Chemical Functionality or Chemical Composition
Pararosaniline base (42500)[‡]	25.6 ± 4.7	Amino-substituted triphenylmethane
Payne's Grey	1.0 ± 0.1	Alizarin lake plus prussian blue plus lampblack plus ultramarine blue
Permanent Magenta	1.1 ± 0.1	Quinacridone
Permanent Rose	2.0 ± 0.1	Quinacridone
Prussian Blue	0.7 ± 0.2 1.6 ± 0.3	Ferric ferrocyanide
Prussian Green	0.9 ± 0.2	Arylamide yellow plus prussian blue
Purple Lake	2.3 ± 0.3	Alizarin lake
Reactive Blue 2 (61211)[‡]	14.4 ± 1.1	Amino-substituted anthraquinone, sulfonate salt
Rose Carthane (12467)	0.8 ± 0.2	Arylamide (Pigment Red 10) plus xanthene (Pigment Red 90)
Rose Doré	2.0 ± 0.2	Quinacridone plus Yellow 3
Thioindigo Violet (73312)[‡]	1.9 ± 1.2	Chlorinated thioindigo
Winsor Yellow (11680)	0.5 ± 0.2	Arylamide yellow

* On watercolor paper unless otherwise indicated. Color Index (CI) names or CI numbers are given in parentheses.
† Mean ± one standard deviation for triplicate samples calculated from the parameters $L*$, $a*$, and $b*$ measured with the color analyzer.
‡ On Whatman 41 paper.

Source: Grosjean et al. (1993)

1   ozone-induce degradation. When ozone degradation was measured using scanning electron

2   microscopy, the oil house paint and latex coating samples showed erosion above that seen with

3   clean air but only at the highest exposure level. No effects were noted for the automotive paint.

4   The other painted surfaces were not evaluated.

5   Spence et al. (1975) studied the effect of air pollutants and relative humidity on oil based

6   house paint, acrylic latex house paint, acrylic coil coating, and vinyl coil coating under

7   laboratory conditions. Test panels were exposed in weathering chambers equipped with a xenon

8   are light for simulating sunlight to low and high levels of ozone (0.08 and 0.5 ppm), sulfur

1   dioxide (0.03 and 0.5 ppm), and nitrogen dioxide (0.05 and 0.5 ppm) and relative humidity

2   (50 and 90%). Samples were exposed for a total of 1000 h. The exposure cycle consisted of

3   20 min of dew and 20 min of light. The effects of the exposure on the painted surfaces were

4   measured by weight loss and loss in film thickness. The acrylic coil coating had the lowest

5   erosion rate of the surface coatings tested. However, ozone was the only pollutant that had a

6   significant effect on the surface erosion. Sulfur dioxide and relative humidity were significant

7   factors in the erosion of oil base house paints and vinyl coil coating. The findings for acrylic

8   latex house paint were not reported.

9

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