

Oxidant Induced Alteration of Carbohydrate Production and Allocation in Plants¹

Robert L. Heath²

Abstract

Urban air basin produced oxidants, notably ozone, induce a decline in productivity in plants. This loss of productivity is manifested by slower growth, hindered development, lower reproduction rates, impaired ability to resist disease, and other stresses. While many metabolic events have been linked to oxidant exposure, three major shifts have been well-studied: increased production and more rapid turnover of antioxidant systems; production of symptoms similar to a mechanical wounding of the tissue, especially ethylene production; and decline in photosynthesis. Although these processes may be linked metabolically at a fundamental level, the mechanisms leading to a decline in photosynthesis have been shown to directly lower plant productivity. There are two distinct changes in physiology which can directly alter the photosynthetic rate by leaves: a closure of the stomata limiting CO₂ concentration, and a decline in the ability to fix CO₂ within the chloroplast. In many studies it is difficult to discriminate which is more critical and which triggers various effects because in the final analysis, both limit carbon assimilation. The mechanisms of stomatal closure may be linked to the loss of membrane permeability and transport because of oxidation of membrane channels and transport proteins and/or oxidation to an increased sensitivity of the stomata to closure signals, such as internal Ca²⁺ levels or abscisic acid. On the other hand, impairment of the processes of photosynthesis is probably not caused by changes in the light gathering photosystems, but rather by a loss of CO₂ fixing ability induced by a decline in Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) or by an alteration in normal metabolite flow via changes in ionic balance. It is not yet clear which of these mechanism is more critical to each individual plant species under varied environmental conditions. However, the loss of photosynthetic products is only the first step in the loss of productivity. Much evidence indicates that translocation of the fixed carbon is altered by oxidants. Because translocation is very sensitive to energy and carbohydrate status as well as membrane function of the leaf, phloem loading would also be at risk by oxidant exposure. A decline in carbohydrate levels to roots and growing shoot tips would have profound effects upon the plant's ability to grow and respond normally to the integrated effects of the remainder of the environment.

Introduction

Many studies that describe the attack of ozone upon the tissues and physiological processes of green plants have used agriculturally important crops (Heagle 1989; Heath 1980, 1994a, [In press]; Heck and others 1988; Karpinski and others 1993). While native plants in the ecosystem may respond differently to ozone than crop plants, certain fundamentals seem to be constant between species. Thus, some generalities of physiology can be stated with certain confidence. To be sure, we have much more to learn, but the concepts and ideas from basic research adds to our "more practical" knowledge base. Understanding how ecosystems can be protected from pollutants requires the foundations of basic research.

Definitions of Forest Decline

"Forest decline" can be defined as the negative changes within forest ecosystems induced by a degeneration in air quality. Two examples of this decline include the process of sinking to a "weaker" or inferior condition, or a diversion from the "normal" development process. In both cases we are forced to define what is meant by the terms "weaker" and "normal." Studies of "normal" ecosystems are few; thus, normal is not inadequate when defined experimentally. Currently, a "normal" ecosystem is that with very low levels of air pollutants. Finding such an ecosystem is not a simple task (Sandermann and others [In press]). A "weaker" ecosystem is one which is not resistant to attack by biotic or abiotic stresses. The loss of trees in

¹ An abbreviated version of this manuscript was presented at the International Symposium on Air Pollution and Climate Change Effects on Forest Ecosystems, February 5-9, 1996, Riverside, California.

² Professor of Plant Physiology and Biophysics, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124 U.S.A.

the San Bernardino Mountains in southern California is a good example because ozone causes an early loss of foliage, seemingly giving the tree less resources to fight a later attack by bark beetle which ultimately kills the tree (Miller 1992).

The observational definitions of plant “changes” caused by air quality include: [1] visible injury, [2] loss of productivity, [3] inability to withstand other stresses, [4] accelerated senescence, and [5] changes in metabolic pathways. Visible injury is often used as the primary observation of forest decline and includes loss of chlorophyll, necrosis, “water-logging” in deciduous leaves, and production of anthocyanin-containing “spots” (Jacobson and Hill 1970). The loss of productivity has been most easily measured by a decline in timber production in a forest. The inability to withstand other stresses has been found in forests attacked by bark beetles, and an inability to compensate for a lowering of nutrient and water availability are examples of other natural abiotic stress. Accelerated senescence is most easily observed as early loss of needles and crown thinning but can include an altered leaf or root development. Changes in metabolic pathways are the most difficult to observe in the field, yet these observations can most clearly indicate a plant’s ability to respond ultimately to the ozone attack.

Plant Physiology and Ozone

Various hypotheses have been proposed to explain the interaction of plant physiological processes and ozone attack (*table 1*). Although the hypotheses overlap at the fundamental biochemical level, each hypothesis is an independent process, and individual research groups tend to focus on only one at a time. The antioxidant protection hypothesis involves both the amount of each antioxidant present and the ability to produce antioxidants which serve to eliminate ozone so that ozone cannot move into any cellular site where it will induce damage. Ozone

Table 1 — Various hypotheses that explain the modification of plant physiological processes induced by ozone.

General	Specific	Detailed
Antioxidant protection	Superoxide:	<i>Superoxide dismutase</i>
	Hydrogen peroxide	<i>Peroxidases</i>
	Hydroxyl radical	<i>Ascorbate, glutathione</i>
		<i>Tocopherol</i>
Wounding response	Wounding proteins or pathogen response proteins	<i>Chitinase</i> <i>β-glucanase</i>
	Ethylene production	
Loss of photosynthetic capacity	Inappropriate stomatal response	<i>Photo-inhibition of photosystems</i>
	Photosynthetic processes	<i>Loss of carboxylation</i>
		<i>Slowing of translocation</i>
Membrane dysfunction	Loss of ion channels (K ⁺ , Ca ²⁺)	
	Loss of permeability	<i>Channels</i>
		<i>Membrane structure</i>
	Loss of signal transduction receptors	

itself is not expected to be stable within the cell or its wall because of its high reactivity. Three major transformation species are currently believed to be formed: superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^\cdot$) (Grimes and others 1983, Möller 1989, Mudd [In press]). Other possibilities, such as ozonide radical ($O_3^\cdot$) and peroxy radical ($HOO^\cdot$) have been described (Heath 1986), but their reactions are currently poorly understood. Data seem to indicate that many oxidative species from ozone can be detoxified easily by reactions with ascorbic acid or glutathione (Benes and others 1995, Bors and others 1989, Chameides 1989, Gupta and others 1991, Guzy and Heath 1993, Kangasjärvi and others 1994, Luwe and others 1993, Polle and others 1995, Willekons and others 1994).

If interception by antioxidants is unsuccessful, then ozone (or its oxidative products) will alter the membrane function through oxidation of critical sulfhydryls of ion channels or pumps and induce an increase in general membrane permeability via inhibition of channel closure or alterations in the membrane structure (Heath 1988, 1994a). Another, but unproved, mechanism is the false triggering of the signal transduction receptors by a direct or indirect chemical modification. Both effects would lead to a non-natural balance of ions across all membranes. The most critical of these ions is Ca^{2+} ; its plasma membrane efflux pump is inhibited and its general permeability is increased by ozone (Castillo and Heath 1990). Many metabolic processes are in turn activated as the Ca^{2+} concentration within the cell rises.

One major response of ozone exposure mimics a general wounding, either mechanical or pathogen induced. Since one of the initial triggers of wounding responses is a rise of Ca^{2+} level within the cell, wounding fits well with what is known about ozone attack. This rise in Ca^{2+} level, in turn, triggers a series of metabolic cascades, ultimately generating ethylene and the production of pathogen-response (PR) proteins (Fengmeier and others 1994, Kärenlampi and others 1994, Langebartels and others 1991, Schraudner and others 1992, Sharma and Davis 1994). It is not clear that ozone directly produces these responses; some oxidative product of ozone may be the key chemical. For example, the prevention of ethylene release has been shown to prevent the induction of visible injury (Mehlhorn and Wellburn 1987, Mehlhorn and others 1991), and the visible injury ultimately has been considered a result of the chemical interaction of ozone and the double bond of ethylene producing a toxic product (Gunderson and Taylor 1991, Taylor and others 1988, Tingey and others 1976).

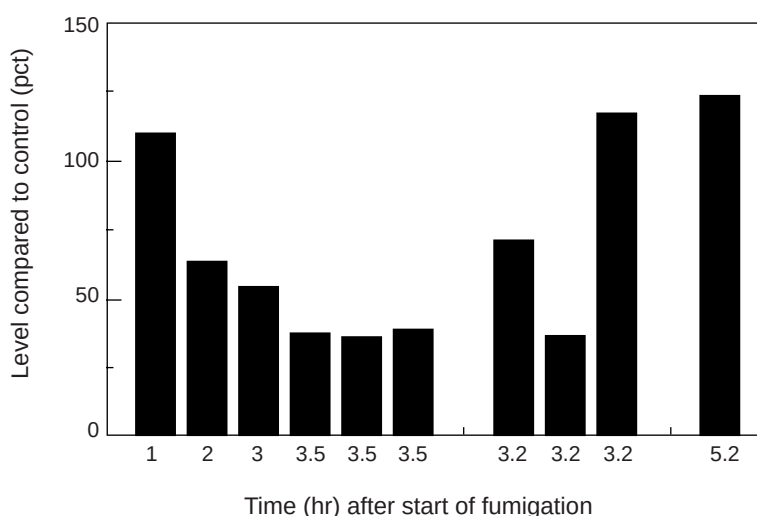
A clear, but mechanistically confusing, plant response to ozone is the loss of photosynthetic capacity. Stomata generally partially close during ozone exposure but, under some conditions, can open further (Heath 1994b). Under many conditions the level of the primary enzyme of CO_2 fixation (ribulose 1,5-bisphosphate carboxylase/oxygenase, Rubisco) declines (Dann and Pell 1989, Nie and others 1993, Pell and others 1994). Yet one clear visible injury pattern, the loss of chlorophyll, signals a problem within the photosystems of the chloroplast (Heath 1989). Photoinhibition can be induced if CO_2 fixation becomes too slow for a given rate of photon capture. Many observed events suggest that while carbon assimilation within the leaf declines, translocation of carbon is inhibited even more so such that growing points of the plant are inhibited and root/shoot ratios are altered (Dugger and Ting 1970, Tjoelker and others 1995).

Examination of the changes in photosynthetic capacity suggests that a multiple series of declines are at the heart of a loss in productivity and resource accumulation of the plant. In general, stomata partially close during ozone exposure. The apertures of stomata are governed by a dynamic balance of water loss and internal CO_2 concentration (which is fixed by a balance of gas flow through the stomata and carbon assimilation) (Farquhar and Sharkey 1982). We do not know if a membrane imbalance first leads to a loss of osmotically accumulated water from the guard cell or if the imbalance is caused by an inhibition of CO_2 fixation. In fact, there are many possible interactive mechanisms: stomata closure can occur with an influx of Ca^{2+} into the guard cell in which its higher concentration changes the sensitivity of the

guard cell to abscisic acid (ABA) transported to the leaf from the roots or produced within the leaf due to a lowered cell water potential (Atkinson and others 1990). These interactive mechanisms make understanding of the full process difficult.

A poor balance between light gathering (photosystems) and CO₂ fixation by Rubisco seems to lead to photoinhibition (Farage and others 1991). Changes in chlorophyll fluorescence patterns and in kinetics of protein production (e.g., the D1 protein of photosystem II) (Barber and Anderson 1992, Osmond 1981) suggest that this is a real mechanism with the possibility that it is not expressed in all plants (Nie and others 1993, Pino and others 1995). The loss of Rubisco is difficult to measure as Rubisco is present in very high concentration. Its level declines in senescing leaves much faster after ozone exposure, suggesting a role in early senescence. An easier method to follow Rubisco changes is by measuring the concentration of its m-RNA leading to the production of the protein *rbcS* (the message for its small subunit is nuclear transcribed). According to studies by Pell and others (1994) and Reddy and others (1993), a rapid, but not complete, loss of the protein message occurs within an hour or so of exposure but recovers within a day after the exposure ceases (fig. 1).

Figure 1 — Changes in the production of m-RNA for the small subunit of Rubisco. Tomato plants, grown in a growth chamber for 3 weeks, were fumigated with 0.33 ppm ozone (produced within O₂ stream) at an air temperature of 28 degrees C and a relative humidity of 25 percent for 3 hours. No visible injury could be seen even after several days, but a slight (ca. 40 percent) stomatal closure could be observed towards the end of the fumigation period (fig. 3). The message RNA was probed (Cohen and Bray 1990) and quantified on the slot blot membrane by the phosphor Imager System (Molecular Dynamics).³



The full scheme for the interaction between membrane dysfunction and Ca²⁺ changes and the other events observed during ozone exposure is not easily decoded. However, the current data indicates that metabolic pathways are regulated and mutually dependent. When ozone alters one metabolic event many others far removed from that initial site of interaction are changed.

We have used a dual label isotope porometer (Johnson and others 1979) to measure the stomata conductance of water vapor (using inward flowing ³H₂O as an analog for normal outward flow of water vapor from the leaf). Assimilation is measured simultaneously by ¹⁴CO₂ fixation. The gas stream passing over the leaf has both isotopes (fig. 2). Knowing the stomata conductance for H₂O vapor allows the calculation of the conductance for O₃ and with the known external level of ozone, a dose of ozone within the leaf can be calculated (Heath 1994b). The inhibition of stomata conductance is greater than the inhibition of assimilation, but under the low light intensity of these experiments, the stomata can close a great deal without inhibiting assimilation (Heath 1994b). We are currently using the dual label porometer in the single label mode (using only ¹⁴CO₂ at high specific activity) to label photosynthetically-fixed carbon in a leaf in order to follow its movement through translocation. Preliminary results suggest ozone exposure dramatically slows carbon movement.

Another method to continuously measure stomata conductance involves the use of leaf temperature as a probe of evaporative water loss through transpiration.

³ Mention of trade names or products is for information only and does not imply endorsement by the U.S. Department of Agriculture.

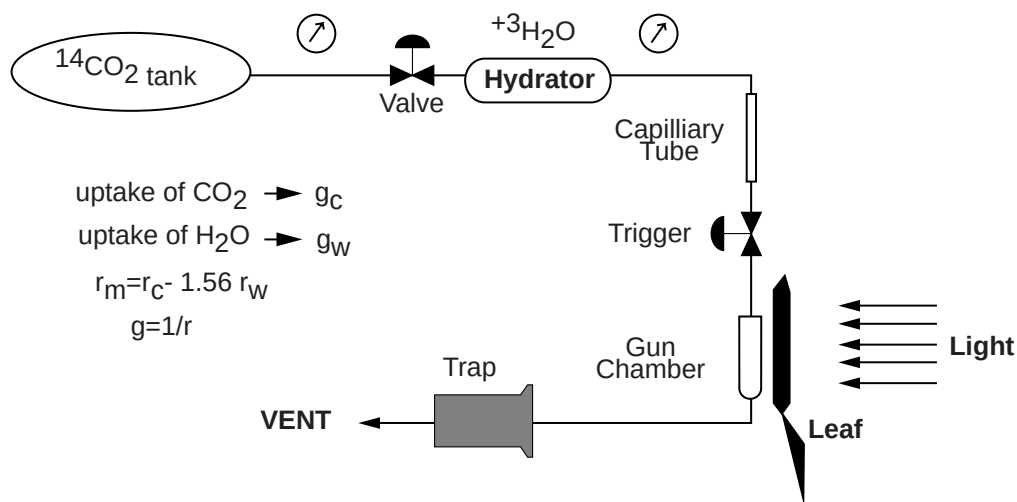
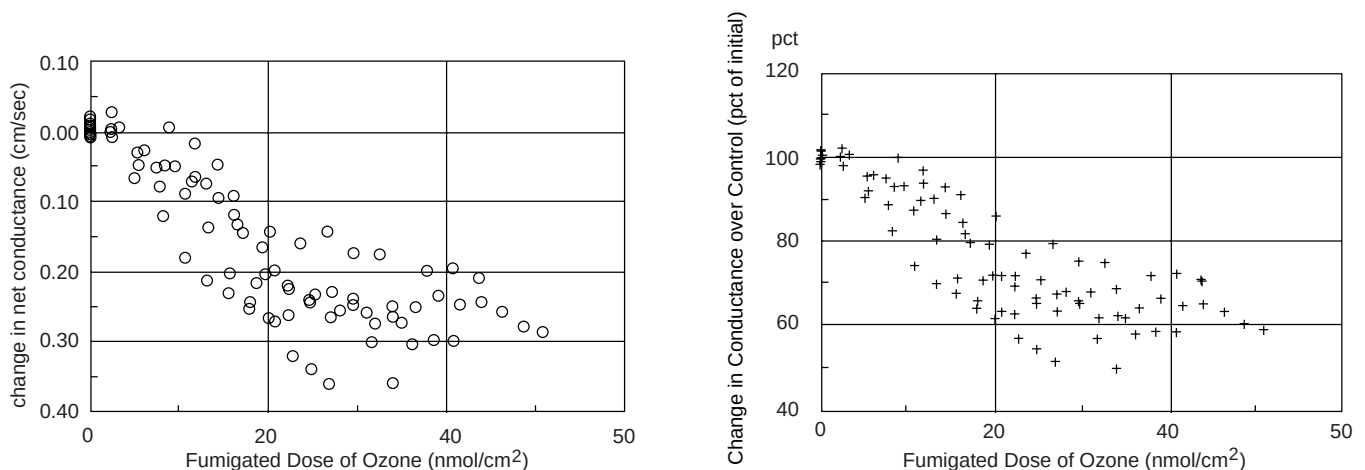


Figure 2 — Dual isotope label porometer (Johnson and others 1979). This machine allows the measurement of water conductivity (measured inversely by the flow of tritiated water from the gas stream into the leaf) and carbon assimilation (measured by the flow of ^{14}C carbon dioxide into the leaf). The area labeled is a circle of diameter of 0.55 cm.

By using two thermocouples wired in series but with legs reversed (the same metal joined at a common junction) and one thermocouple touching the leaf and the other in the air just below the leaf, the differential temperature ($dT = T_{\text{leaf}} - T_{\text{air}}$) can be continuously recorded. Calibrations allows dT to be used as a measure of transpiration rate. By using the air temperature and relative humidity, the stomata conductance can be calculated, and the ozone dose can be “measured” continuously during exposure. A plot for tomato exposed to ozone was measured by using this technique (*fig. 3*). The change in conductance is measured against the actual delivered dose of ozone to six leaves. Each leaf begins with a slightly different conductance (as they are different leaves on the two plants and are at different developmental ages, which affects their conductance); thus, the total time of exposure at which stomata begin to close varies. However, converting time into accumulated dose for each gives clearer results; the dose for the beginning of closure is about the same as $8 \text{ nmole cm}^{-2}\text{-leaf area}$. The stomata close to about 50-60 percent of the initial level at a dose of about $30\text{-}35 \text{ nmole cm}^{-2}\text{-leaf area}$. Higher doses do not seem to close the stomata any further.



Conclusion

Figure 3 — Changes of stomatal conductance during fumigation of tomato plants with ozone. The data were collected and dose was calculated by the amount of ozone in the air multiplied by the stomatal conductance for ozone each 210 seconds. This is an average of thermocouple measurements for six leaves (2 on 2 plants, 1 on 4 plants). Several plants were destructively removed during the course of the experiment for m-RNA sampling.

Results of the various studies discussed in this paper have made sizable advancement in the understanding of ozone induced plant changes. Several testable hypotheses regarding the mechanism of ozone induced injury have been formulated (Bors and others 1989, Castillo and Heath 1990, Eckardt and Pell 1994, Langebartels and others 1991, Pino and others 1995) and cultivars and mutants of varied species have been generated which differ in their sensitivities to ozone (Guzy and Heath 1993, Sharma and Davis 1994). The understanding of the basic processes of plants has made this progress possible. Our comprehension of how plants respond to the environment continues to grow. In the future, better technology to allow us to measure physiological events, and a more complete understanding of genetics and the use of mutants should make it possible to understand how ozone exposure puts a plant at risk and how we can aid the plant in its attempt to protect and repair itself in the face of that risk.

References

- Atkinson, C.J.; Mansfield, T.A.; McAinsh, M.R.; Brownless, C.; Hetherington, A.M. 1990. **Interactions of calcium with abscisic acid in the control of stomatal aperture.** *Biochemical Physiology Pflanzen* 186: 333-339.
- Barber, J.; Andersson, B. 1992. **Too much of a good thing: light can be bad for photosynthesis.** *Trends in Biological Science* 17: 61-66.
- Benes, S.E.; Murphy, T.M.; Anderson, P.D.; Houpi, J.L.J. 1995. **Relationship of antioxidants enzymes to ozone tolerance in branches of mature ponderosa pine (*Pinus ponderosa*) trees exposed to long-term, low concentration, ozone fumigation and acid precipitation.** *Physiologia Plantarum* 94: 123-134.
- Bors W.; Langebartels, C.; Michel, C.; Sandermann, Jr., H. 1989. **Polyamines as radicals scavengers and protectants against ozone damage.** *Phytochemistry* 28: 1589-1595.
- Castillo, F.J.; Heath, R.L. 1990. **Ca²⁺ Transport in membrane vesicles from pinto bean leaves and its alteration after ozone exposure.** *Plant Physiology* 94: 788-795.
- Castillo, F.J.; Miller, P.R.; Greppin, H. 1987. **Extracellular biochemical markers of photochemical oxidant air pollution damage to Norway spruce.** *Experientia* 43: 111-115.
- Chameides, W.L. 1989. **The chemistry of ozone deposition to plant leaves: Role of ascorbic acid.** *Environmental Science and Technology* 23: 595-600.
- Cohen, A.; Bray, E.A. 1990. **Characterization of three mRNAs that accumulate in wilted tomato leaves in response to elevated levels of endogenous abscisic acid.** *Planta* 182: 27-33.
- Dann, M.S.; Pell, E.J. 1989. **Decline of activity and quantity of Ribulose Bisphosphate Carboxylase/Oxygenase and net photosynthesis in O₃-treated potato foliage.** *Plant Physiology* 91: 427-432.
- Dugger, W.M.; Ting, I.P. 1970. **Air pollution oxidants—Their effects on metabolic processes in plants.** *Annual Review of Plant Physiology* 21: 215-234.
- Eckardt, N.A.; Pell, E.J. 1994. **O₃-induced degradation of Rubisco protein and loss of Rubisco mRNA in relation to leaf age in *Solanum tuberosum* L.** *New Phytologist* 127: 741-748.
- Fangmeier, A.; Brunschön, H.J.; Jäger, H.J. 1994. **Time course of oxidant stress biomarkers in flag leaves of wheat exposed to ozone and drought stress.** *New Phytologist* 125: 63-69.
- Farage, P.K.; Long, S.P.; Lechner, E.G.; Baker, N.R. 1991. **The sequence of changes within the photosynthetic apparatus of Wheat following short term exposure to ozone.** *Plant Physiology* 95: 529-535.
- Farquhar, G.D.; Sharkey, T.D. 1982. **Stomatal conductance and photosynthesis.** *Annual Review of Plant Physiology* 33: 317-345.
- Grimes, H.D.; Perkins, K.K.; Boss, W.F. 1983. **Ozone degrades into hydroxyl radical under physiological conditions.** *Plant Physiology* 72: 1016-1020.
- Gunderson, C.A.; Taylor, Jr., G.E. 1991. **Ethylene directly inhibits foliar exchange in *Glycine max*.** *Plant Physiology* 95: 337-339.
- Gupta, A.S.; Alscher, R.G.; McCune, D. 1991. **Response of photosynthesis and cellular antioxidants to ozone in *Populus* leaves.** *Plant Physiology* 96: 650-655.
- Guzy, M.R.; Heath, R.L. 1993. **Response to ozone of varieties of common bean (*Phaseolus vulgaris* L.)** *New Phytologist* 124: 617-625.
- Heagle, A.S. 1989. **Ozone and crop yield.** *Annual Review Phytopathology* 27: 397-423.
- Heath, R.L. 1980. **Initial events in injury to plants by air pollutants.** *Annual Review of Plant Physiology and Plant Molecular Biology* 31: 395-431.
- Heath, R.L. 1987. **The biochemistry of ozone attack on the plasma membrane of plant cells.** *Advanced Phytochemistry* 21: 29-54.
- Heath, R.L. 1988. **Biochemical mechanisms of pollutant stress.** In: Heck, W.W.; Taylor, O.C.; Tingey, D.T., eds *Assessment of crop loss from air pollutants.* London: Elsevier Applied Science; 259-286.
- Heath, R.L. 1989. **Alteration of chlorophyll in plants upon air pollutant exposure.** In: Woodwell, G.M.; Cook, E.R.; Cowling, E.B.; Johnson, A.H.; Kimmerer, T.W.; Matson, P.A.; McLaughlin, S.S.; Raynal, D.J.; Swank, W.T.; Waring, R.H.; Winner, W.E.; Woodman, J.N., eds *Biologic markers of air-pollution stress and damage in forests.* Washington, D.C.: U.S. National Academy Press; 347-356.

- Heath, R.L. 1994a. **Alterations of plant metabolism by ozone exposure.** In: Alscher, R.G.; Wellburn, A.R., eds. Plant responses to the gaseous environment. London: Chapman and Hall; 121-146.
- Heath, R.L. 1994b. **Possible mechanisms for the inhibition of photosynthesis by ozone.** Photosynthesis Research 39: 439-451.
- Heath, R.L. [In press]. **The modification of photosynthetic capacity induced by ozone exposure.** In: Baker, N.R., ed. Photosynthesis and the environment. Advances in Photosynthesis. Amsterdam: Kluwer Academic Publishers.
- Heck, W.W.; Taylor, O.C.; Tingey, D.T. 1988. **Assessment of crop loss from air pollutants.** London: Elsevier Applied Sciences; 552 p.
- Jacobson, J.S.; Hill, A.C. 1970. **Recognition of air pollution injury to vegetation: a pictorial atlas.** Pittsburg, PA: Air Pollution Control Association; 150 p.
- Johnson, H.B.; Rowlands, P.G.; Ting, I.P. 1972. **Tritium and carbon-14 double isotope porometer for simultaneous measurements of transpiration and photosynthesis.** Photosynthetica 13: 409-418.
- Kangasjärvi, J.; Talvinen, J.; Utriainen, M.; Karjalainen, R. 1994. **Plant defense systems induced by ozone.** Plant Cell and Environment 17: 783-794.
- Kärenlampi, S.O.; Airaksinen, K.; Miettinen, A.T.E.; Kokko, N.I.; Holopainen, J.K.; Kärenlampi, L.V.; Karjalainen, R.O. 1994. **Pathogenesis-related proteins in ozone exposed Norway Spruce [*Picea abies* (Karst) L.].** New Phytologist 126: 81-89.
- Karpinski, S.; Wingsle, G.; Karpinski, B.; Hällgren, J.-E. 1993. **Molecular responses to photooxidative stress in *Pinus sylvestris* (L.)** Plant Physiology 103: 1385-1391.
- Langebartels, C.; Kerner, K.; Leonard, S.; Schraudner, M.; Trost, M.; Heller, W.; Sandemann, Jr., H. 1991. **Biochemical plant response to ozone I. Differential induction of polyamine and ethylene biosynthesis in Tobacco.** Plant Physiology 95: 882-889.
- Luwe, M.W.F.; Takahama, U.; Heber, U. 1993. **Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia oleracea* L.) leaves.** Plant Physiology 101: 969-976.
- Mehlhorn, H.; O'Shea, J.M.; Wellburn, A.R. 1991. **Atmospheric ozone interacts with stress ethylene formation by plants to cause visible plant injury.** Journal of Experimental Botany 42: 17-24.
- Mehlhorn, H.; Wellburn, A.R. 1987. **Stress ethylene formation determines plant sensitivity to ozone.** Nature 327: 417-418.
- Miller, P.R. 1992. **Mixed conifer forests of the San Bernardino mountains, California.** In: Olson, R.K.; Binkley, D.; Bohm, M., eds. The response of western forests to air pollution. New York: Springer-Verlag; 461-497.
- Möller, D. 1989. **The possible roles of H₂O₂ in new type forest decline.** Atmospheric Environment 23: 1625-1627.
- Mudd, J.B. [In press]. **Biochemical basis for the toxicity of ozone.** In: Iqbal, M.; Yunus, M., eds. Plant response to air pollution. Chichester, UK: John Wiley and Sons Ltd.
- Nie, G.-Y.; Tomasevic, M.; Baker, N.R. 1993. **Effects of ozone on the photosynthetic apparatus and leaf proteins during leaf development in wheat.** Plant Cell and Environment 16: 643-651.
- Osmond, C.B. 1981. **Photorespiration and photoinhibition: some implications for the energetics of photosynthesis.** Biochimica et Biophysica Acta 639: 77-98.
- Pell, E.J.; Eckardt, N.; Glick, R.E. 1994. **Biochemical and molecular basis for impairment of photosynthetic potential.** Photosynthesis Research 39: 453-462.
- Pino, M.E.; Mudd, J.B.; Bailey-Serres, J. 1995. **Ozone-induced alterations in the accumulation of newly synthesized proteins in leaves of maize.** Plant Physiology 108: 777-785.
- Polle, A.; Wieser, G.; Havranek, W.M. 1995. **Quantification of ozone influx and apoplastic ascorbate content in needles of Norway Spruce trees (*Picea abies* L. Karst) at high altitude.** Plant Cell and Environment 18: 681-688.
- Reddy, G.N.; Arteca, R.N.; Dai, Y.R.; Flores, H.E.; Negm, F.B.; Pell, E.J. 1993. **Changes in ethylene and polyamines in relation to mRNA levels of the large and small subunits of ribulose biphosphate carboxylase/oxygenase in ozone-stress potato foliage.** Plant Cell and Environment 16: 819-826.
- Sandermann, Jr., H.; Wellburn, A.; Heath, R.L., eds. [In press]. **Forest decline and ozone: a comparison of controlled chamber and field experiments.** Berlin: Springer-Verlag.
- Schraudner, M.; Ernst, D.; Langebartels, C.; Sandermann, Jr., H. 1992. **Biochemical plant responses to O₃ III: Activation of the defense-related proteins -1,3-glucanase and chitinase in tobacco leaves.** Plant Physiology 99: 1321-1328.
- Sharma, Y.K.; Davis, K.R. 1994. **Ozone-induced expression of stress-related genes in *Arabidopsis thaliana*.** Plant Physiology 105: 1089-1096.
- Taylor, Jr., G.E.; Ross-Todd, B.M.; Gunderson, C.A. 1988. **Action of ozone on gas exchange in *Glycine max* L. Merr: A potential role for endogenous stress ethylene.** New Phytologist 110: 301-307.
- Tingey, D.T.; Standley, C.; Field, R.W. 1976. **Stress ethylene evolution: A measure of ozone effects on plants.** Atmospheric Environment 10: 969-974.
- Tjoelker, M.G.; Volin, J.C.; Oleksyn, J.; Reich, P.B. 1995. **Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment.** Plant Cell and Environment 18: 895-905.
- Willekens, H.; Van Camp, W.; Van Montagu, M.; Inzé, D.; Langebartels, C.; Sandermann, Jr., H. 1994. **Ozone, sulfur dioxide, and ultraviolet B have similar effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia* L.** Plant Physiology 106: 1007-1014.

