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Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning

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ABSTRACT

The inhibitory effects of tropospheric O_3 on crop photosynthesis, growth, and yield have been documented in numerous studies over the past 35 years. In large part, the results of this research supported governmental regulations designed to limit tropospheric O_3 levels to concentrations that affected crop production at economically acceptable levels. Recent studies have brought into question the efficacy of these concentration-based O_3 standards compared with flux-based approaches that incorporate O_3 uptake along with environmental and biotic factors that influence plant responses. In addition, recent studies provide insight into the biochemical mechanisms of O_3 injury to plants. Current interpretations suggest that upon entry into the leaf intercellular space O_3 rapidly reacts with components of the leaf apoplast to initiate a complex set of responses involving the formation of toxic metabolites and generation of plant defence responses that constitute variably effective countermeasures. Plant species and cultivars exhibit a range of sensitivity to O_3, evident as heritable characteristics, that must reflect identifiable biochemical and molecular processes that affect sensitivity to O_3 injury, although their exact makeup remains unclear. Ozone clearly impairs photosynthetic processes, which might include the effects on electron transport and guard cell homeostasis as well as the better-documented effects on carbon fixation via decreased Rubisco activity. Translocation of photosynthate could be inhibited by O_3 exposure as well. Further, the influence of tropospheric O_3 needs to be considered when assessing potential effects of rising concentrations of atmospheric CO_2 on crop production. Advances in O_3 flux modelling and improved understanding of biochemical and molecular effects of O_3 on photosynthetic gas exchange and plant defence processes are leading to more complete, integrated assessments of O_3 impacts on crop physiology that continue to support the rationale for maintaining or improving current O_3 air quality standards as well as providing a basis for development of more O_3-tolerant crop lines.

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INTRODUCTION

Tropospheric O_3 is currently viewed as a widespread and growing problem that suppresses crop productivity on a large scale (US Environmental Protection Agency 1996; Mauzerall & Wang 2001; Fuhrer & Booker 2003). The problem was originally perceived to be limited in nature, confined to urban centres, proximity to power plants, and areas downwind or nearby these sources. However, the scale of the problem has increased in scope in the last 25 years as a result of increasing population densities, industrialization, and transportation-related activities in large parts of the world, particularly in the less developed countries. In addition there is increased recognition of transboundary transport of O_3 precursors in the troposphere. In response, a number of efforts have been made to define the impact of O_3 on crop productivity, use this information to predict crop losses, and to set air quality standards that should keep crop losses to an economically acceptable level (US Environmental Protection Agency 1996; UNECE 2000).

Current worldwide average tropospheric O_3 levels were approximately 50 nmol mol^{-1} in the year 2000, already 25% above the threshold established for damage to sensitive plants (Fuhrer, Skarby & Ashmore 1997). While global mean values have increased from an estimated pre-industrial level of 38 nmol mol^{-1} [25–45 nmol mol^{-1}, 8-h summer seasonal average (US Environmental Protection Agency 1996)] to about 50 nmol mol^{-1} in 2000, the most pessimistic projections suggest a further increase to 80 nmol mol^{-1} by 2100. Most of this increase would be driven by a nearly three-fold increase in NO_X and CH_4 emissions (Prather *et al*. 2001). It is critical to understand that these global means are comprised of local means, many of which are already substantially above the projections and typically occur sometime during the cropping season.

Although the actual economic costs of O_3-induced crop losses are difficult to assess, the total benefits resulting from various regulatory scenarios, mostly involving reductions of current ambient levels, ranged from about 0.1–2.5 B$ (in 1980 US dollars) in the United States (Adams & Horst 2003). Additionally, the US Environmental Protection

Agency in its 1996 criteria document estimated annual national level losses to major crops to be in excess of 1 B$ (in 1990 US dollars) (US Environmental Protection Agency 1996). Further, Mauzerall & Wang (2001) cite several recent studies estimating benefits of 2–3.3 B$ in the US by eliminating O_3 precursors from motor vehicle emissions (Murphy *et al*. 1999), and 310 M€ in the Netherlands (Kuik *et al*. 2000), and 2 B$ in China (Mauzerall & Wang 2001) by reducing O_3 to background levels (see also Ashmore 2005; this issue). Also, due to the non-linear shape of many crop-O_3 dose–response curves, we might expect a disproportionately larger effect for each unit increase in global average O_3 concentrations.

The yield or productivity responses to O_3 of a wide range of crops clearly show a negative relationship, and there seems little to be gained by dwelling further on that issue except to emphasize that a wide range of responses and levels of sensitivity exists among the many plants that have been tested (Heck *et al*. 1983; Heagle 1989; US Environmental Protection Agency 1996; Fuhrer *et al*. 1997; Morgan, Ainsworth & Long 2003). The past decade has seen substantial progress in interpreting the effects of O_3 on plants and the mechanisms by which those effects are mediated. However, there is still much to be done in a practical sense before that information can be translated into useful products.

It is our purpose in this paper to review recent progress in understanding how O_3 brings about its effects and to raise occasional questions or note exceptions to the current dogma. We start by examining the rationale for flux-based characterizations of O_3 exposures, which may prove advantageous for formulation and implementation of environmental impact assessment and regulation. Then we consider O_3 entry into leaves, and the immediate reactions that occur in the leaf apoplast, the protective mechanisms that may be activated, and possible signals generated that result in modified plant behaviour. Then, since photosynthesis seems consistently affected by O_3 exposure, especially during the reproductive phase of crop development, and since photosynthetic processes directly contribute to crop productivity, we review some recent evidence detailing the effects of O_3 exposure on photosynthetic systems in various crop species. In addition, the distribution of the resultant photosynthate is considered in relation to O_3 stress and how perturbations in that distribution might contribute to the overall impact. Potential interactions between rising concentrations of atmospheric CO_2 and O_3 are briefly addressed. Finally we touch upon some of the sources of experimental variability and the consequences thereof.

O_3 EXPOSURE AND UPTAKE

First interpretations of cause and effect relationships between O_3 and plant responses are commonly based on the O_3 concentrations in the surrounding air. For example, various plant responses to O_3 have been correlated with average O_3 concentration over some time interval (e.g. daily 7, 8, or 12 h averages). Other, more biologically relevant schemes, illustrated in Fig. 1, involve summing O_3 concentrations that exceed some threshold value expressed either as a step function (e.g. SUM06, AOT40), or a continuous sigmoidal weighting function (e.g. W126). Still others involve a combination of hourly averages and number of peak values (Lefohn 1992; US Environmental Protection Agency 1996). The advantages of concentration-based approaches are clear: measurements of O_3 concentration and computation of exposure indices are straightforward, and air quality standards can be based on verifiable data. The validity of this approach is supported by numerous controlled environment, greenhouse, and field experiments that show a consistent negative relationship between O_3 exposure and photosynthesis, growth, and yield (Miller 1987b; Heagle 1989; Fuhrer *et al*. 1997).

Atmospheric O_3 concentration indices are used worldwide for establishing air quality standards. Experimental results, however, indicate a wide range in relative tolerance to O_3 among crops species and cultivars, which is influenced by a number of environmental factors and other air pollutants experienced during plant growth (US Environmental

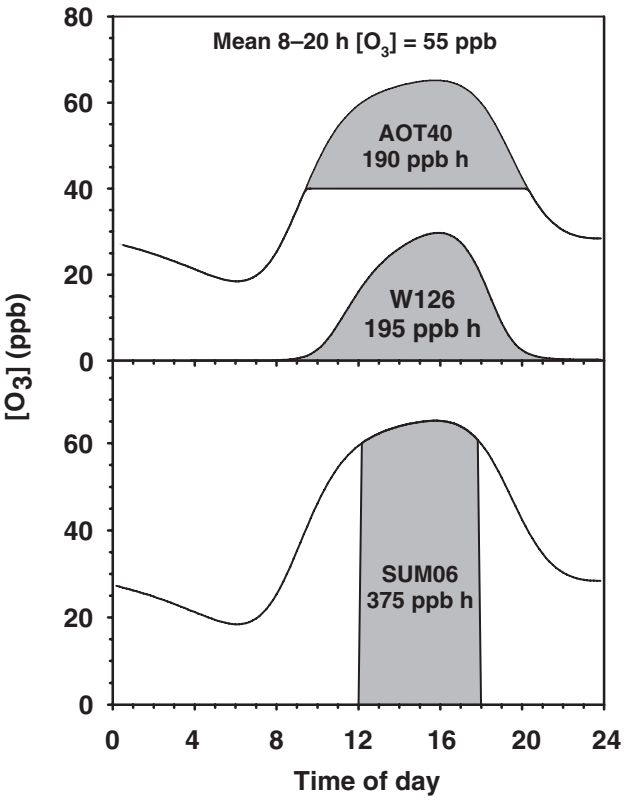

Figure 1. Illustration of the SUM06, AOT40, and the continuous sigmoidal weighting scheme imposed on a typical summertime daily average $[O_3]$ curve for Raleigh, NC, USA. Each of the functions use hourly average $[O_3]$ and are defined as: AOT40 = $\Sigma([O_3]–40)$ for $[O_3] > 40$; SUM06 = $\Sigma[O_3]$ for $[O_3] \geq 60$; and W126 = $\Sigma([O_3]\,W)$, where the sigmoidal weighting function $W = 1/(1 + 4403(\exp(–0.126\,[O_3])))$ (US Environmental Protection Agency (1996). All concentrations are in ppb or the SI equivalent and W126 is applied to all the hourly averages. Units for all are ppbh.

Protection Agency 1996; Fuhrer *et al*. 1997). In addition, the O_3 concentration at measuring height (3–10 m) is usually not corrected for the lower concentration received at canopy height (Tuovinen 2000). However, a protocol for making this correction has been devised (UNECE 2004b).

Efforts to understand the discrepancies between O_3 concentration in the air and variable plant responses have focused on molecular and biochemical mechanisms involved in O_3 detoxification processes and certain interacting environmental factors affecting stomatal conductance (g_s) (Fuhrer *et al*. 1997; Massman 2004; Pleijel *et al*. 2004). Environmental factors influencing g_s are important because O_3 uptake mainly occurs through leaf stomata (Runeckles 1992; Long & Naidu 2002). Various modelling results have indicated that a major factor affecting plant responses to O_3 appears to be g_s (Runeckles 1992; Massman 2004; Pleijel *et al*. 2004). This is supported by experiments with a variety of crops exposed simultaneously to O_3 and elevated atmospheric CO_2, an effective antitranspirant, that showed reduced O_3 injury, presumably due in large part to lowered O_3 flux (McKee, Farage & Long 1995; Fiscus *et al*. 1997; Reid & Fiscus 1998; Reid, Fiscus & Burkey 1998; Heagle *et al*. 1999; Reid, Fiscus & Burkey 1999; Olszyk *et al*. 2000; Fiscus *et al*. 2002; Morgan *et al*. 2003; Booker, Fiscus & Miller 2004; Booker *et al*. 2005). Reduced O_3 damage to water-stressed soybean [*Glycine max* (L) Merr.] compared with well-watered plants was attributed to lower g_s as well (Tingey & Hogsett 1985; Vozzo *et al*. 1995).

The major environmental factors controlling g_s include leaf temperature, water vapour pressure difference between the leaf and the surrounding air (VPD), photosynthetic photon flux density (PPFD), soil water availability, and atmospheric CO_2 concentration (Jarvis 1976). Thus, responses to some O_3 concentration for a particular crop and cultivar undoubtedly depend upon inherent genetic differences and environmental conditions experienced during the growing season. In order to incorporate the effects of environmental conditions relevant to g_s into an exposure index, the UNECE ICP Vegetation project is investigating the use of VPD estimates in conjunction with O_3 concentration for defining the critical O_3 level that produces visible injury in crops (UNECE 2004b). Stomatal conductance is typically higher in warm, humid environments compared with hot, dry conditions. Thus, crops in warm, humid environments are likely at increased risk of O_3 injury. This approach holds promise as a relatively simple method for including environmental factors that influence g_s into O_3 exposure indices. However, other factors such as leaf temperature, leaf hydration, or PPFD (Massman 2004) could independently affect O_3 toxicity, which would require additional modifications of such an index.

From a toxicological viewpoint, it is the absorbed cumulative dose of O_3 that is most relevant in determining cause and effect relationships and quantifying dose–responses. Thus, O_3 flux models using a multiplicative algorithm based on the approach of Jarvis (1976) to model g_s have incorporated VPD, air temperature, PPFD, and soil moisture deficit coefficients to compute estimates of O_3 uptake (Emberson *et al*. 2000; Pleijel *et al*. 2004). A phenological component also has been added to account for changes in g_s during the growing season. This approach indicated that phenology and VPD were important factors in estimating O_3 flux. Comparisons between flux estimates and O_3 exposure maps of Europe suggested that areas with the highest O_3 concentrations differed from those regions calculated as having the highest O_3 fluxes (Emberson *et al*. 2000). The highest O_3 concentrations occurred in the southern Mediterranean region whereas the highest O_3 fluxes for wheat occurred in southern Scandinavia and northern Europe.

However, some crops and cultivars with similar g_s have different tolerances to the same O_3 concentrations. Thus, it has been suggested that O_3 flux models include a coefficient for O_3 detoxification capacity (Musselman & Massman 1999; Fuhrer & Booker 2003; Massman 2004). Musselman & Massman (1999) defined the 'effective flux' as the balance between uptake into the leaf at a given time and the defence response at that time. The defence response factor was proportional to the effect of O_3 on gross photosynthesis. Effective flux integrated over time yields cumulative effective loading. Using a relatively comprehensive modelling approach for O_3 deposition and uptake, Massman (2004) estimated that the period of highest effective loading occurred between 1300 and 1600 h.

The cumulative exposure indices (SUM06, W126, AOT40) are based on O_3 injury thresholds for effects of O_3 concentration on plant growth and yield. In the same manner, cumulative O_3 flux models show improved performance when a threshold O_3 flux factor is included in the model, e.g. Pleijel *et al*. (2004). Likewise, Martin *et al*. (2000) modified WIMOVAC (Windows Intuitive Model of Vegetation Response to Atmosphere and Climate Change) (Humphries & Long 1995) to include O_3 detoxification processes when calculating threshold flux-based, rather than concentration-based, dose–response models. However, determination of the threshold coefficient in the Martin *et al*. (2000) model requires experimentally derived assessments of O_3 effects on V_{cmax}. The situation is a bit more complicated because O_3 can damage plant photosynthesis, which in turn may reduce the plant's detoxification ability (Massman 2004). In effect there are interacting mechanisms at work that may alter O_3 injury thresholds as damage accumulates (Massman 2004). Ozone increases the relative cost of detoxification on the one hand and decreases the need, through C_i-driven reductions in g_s, on the other. A flux-based approach that includes thresholds requires further experimentation and advances in our understanding of fundamental processes involved in O_3 action among different plant species. Furthermore, this approach needs to be scaled to the canopy level to be generally applicable. Micrometeorological soil-vegetation-atmosphere transfer (SVAT) models have been developed that estimate stomatal O_3 flux and cuticular deposition based on O_3 concentration, atmospheric, boundary layer and bulk canopy resistances to describe the vertical exchange of O_3 to the plant-soil system (Grünhage, Haenel & Jäger 2000).

In the field, canopy structure and foliage density affect the concentration of O_3 that leaves experience, while environmental factors such as PPFD, leaf temperature and VPD vary within the leaf canopy and can affect g_s and thus O_3 flux. However, after canopy closure for crops such as soybean, cotton (*Gossypium hirsutum* L.), and peanut (*Arachis hypogaea* L.), leaves at the top of the canopy provide the majority of photosynthate for seed production. Ozone flux to the upper portion of the canopy is likely most critical to impacts on yield. For crops such as wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), flux to the entire canopy might be important. Efforts to parameterize canopy O_3 flux have included leaf area index as a way to estimate canopy g_s (Massman 2004), which may be effective in accounting for differences among crop canopy structures. However, the ICP Vegetation protocol utilizes projected leaf area, namely leaf area of sunlit leaves at the top of the canopy, to calculate O_3 flux (Pleijel *et al*. 2004; UNECE 2004b). Ozone flux to the most productive leaves is viewed as having the greatest impact on yield because, even though the soil and lower parts of the canopy consume large amounts of O_3, it is likely these fluxes have much less effect on yield (Pleijel *et al*. 2004; Ashmore 2005).

Lastly, the ICP Vegetation program (UNECE 2004a) is currently comparing economic crop loss assessments of concentration-based and flux-based methodologies. This analysis should suggest whether the flux approach provides a significantly better assessment of crop losses due to O_3 pollution compared with concentration-based exposure indices.

MODES OF ACTION

Apoplastic and symplastic reactions and responses

The internal air spaces within the leaf are a potential site for O_3 reactions with volatile compounds produced by the plant. Isoprene is one example of a compound emitted from many angiosperms and conifers (Sharkey & Yeh 2001) that could react with O_3. However, volatile compounds released by crop species are mainly monoterpenes, which have been little studied in this context although parallels between these volatile organic compounds (VOCs) and O_3 reactions with isoprene are likely. At present, hypotheses are contradictory regarding the role of isoprene in plant responses to O_3. Initially, isoprene was thought to enhance O_3 damage through the formation of hydroperoxides (Hewitt, Kok & Rall 1990). More recently, isoprene was found to exert a protective effect either through action as a radical scavenger or membrane stabilizer (Loreto & Velikova 2001). The concept of isoprene as an antioxidant is intriguing from the perspective of O_3 scavenging reactions outside the leaf that could increase boundary layer resistance to O_3 and effectively reduce the dose. The potential benefits of such a mechanism must be balanced against the metabolic demand for carbon to support isoprene synthesis (Sharkey & Yeh 2001) and the consequences of increased plant-derived VOCs on atmospheric chemistry leading to O_3 formation.

Ozone that has passed through leaf internal air spaces will then dissolve in the aqueous layer surrounding leaf cells. The breakdown of O_3 in pure water produces hydroxyl and peroxyl radicals and superoxide although the reactions proceed very slowly at neutral pH (Heath 1987). Grimes, Perkins & Boss (1983) demonstrated that the rate of hydroxyl radical formation from O_3 in aqueous solution is significantly greater when phenolic compounds are present, suggesting that biologically relevant compounds enhance O_3 reactions. Based on *in vitro* and *in vivo* studies (Heath 1987; Runeckles & Chevone 1992; Pryor 1994; Kirichenko *et al*. 1996), O_3 is known to react with a diverse set of molecules that would be encountered within the cell wall and on the plasma membrane surface. Initial targets for O_3 include plasma membrane lipids, susceptible amino acids in plasma membrane proteins or apoplastic enzymes, and a variety of organic metabolites localized in the cell wall. Potentially, ozonolysis products from the oxidation of lipids and metabolites containing carbon-carbon double bonds (Heath 1987; Runeckles & Chevone 1992) and altered plasma membrane protein function (Dominy & Heath 1985; Castillo & Heath 1990) could serve as the initial signals leading to O_3 responses. However, the primary O_3 reaction products in plants have not been identified due to difficulties in following O_3 reactions within the complex biochemical network of the leaf apoplast. Knowledge of these early events is critical to understanding the metabolic signals that initiate O_3 responses, and may provide insight into O_3 tolerance mechanisms localized in the leaf apoplast. Ozone incorporation into the cells and extracellular lipid-protein complexes of rat lung tissue has been studied using a combination of $^{18}O_3$ and mass spectrometry (Gunnison & Hatch 1999), an approach that could be employed in conjunction with available methods for isolation of leaf apoplast components and plasma membranes to address questions regarding the initial O_3 reaction products in plants.

Formation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radical are thought to be associated with the initial breakdown of O_3 in the leaf apoplast. ESR approaches, based on spin trapping, suggest that free radicals are involved in the early stages of O_3 response (Grimes *et al*. 1983; Mehlhorn, Tabner & Wellburn 1990; Runeckles & Vaartnou 1997), but it is not clear whether the observed signals originate from direct O_3 breakdown or the initial reactions with cellular constituents such as phenolic compounds. Localized regions of ROS formation, either hydrogen peroxide or superoxide depending on the plant species, are associated with O_3 response in sensitive plants (Wohlgemuth *et al*. 2002; Pendall *et al*. 2004). The kinetics of ROS formation in the leaf apoplast following O_3 exposure seem to involve two phases, an initial phase that is associated with direct effects of O_3 and a second phase associated with a plant-derived secondary oxidative burst (Schraudner *et al*. 1998). The secondary oxidative burst is initially localized in the leaf apo-

plast and cell wall, but later expands into the cytoplasm and subcellular compartments leading to the formation of visible lesions (Pendall *et al*. 2004).

The initial signals induced by O_3 in the leaf apoplast might then be translated into responses at the tissue level. Responses vary and include unregulated cell death, hypersensitive response leading to programmed cell death, and accelerated senescence (Pell, Schlagnhaufer & Arteca 1997; Sandermann *et al*. 1998). Evidence is accumulating that these responses are modulated by ethylene, jasmonic, and salicylic acid levels, and the interactions among their signalling pathways (Kangasjärvi *et al*. 1994; Sharma *et al*. 1996; Pell *et al*. 1997; Overmyer *et al*. 2000; Rao *et al*. 2000; Rao & Davis 2001; Rao, Lee & Davis 2002; Overmyer, Brosche & Kangasjärvi 2003; Tamaoki *et al*. 2003; Vahala *et al*. 2003; see also Kangasjärvi *et al*. 2005; this issue). For example, *Arabidopsis thaliana* mutants that overproduce ethylene (*eto1*) or are insensitive to jasmonic acid (*jar1*) are more extensively injured by O_3 than wild-type plants (Overmyer *et al*. 2000; Rao *et al*. 2000; Tamaoki *et al*. 2003). Ethylene acts as a promoting factor for O_3 injury whereas jasmonates might have a role in minimizing injury (Overmyer *et al*. 2000, 2003).

Ozone effects at the tissue level are often characterized as either acute or chronic responses, subjective terms that are related to the type of visible symptoms observed and whether the O_3 concentrations employed typify ambient O_3 episodes. For example, unregulated cell death and programmed cell death are usually considered acute responses in which lesions occur within hours after exposure to relatively high O_3 concentrations (typically >150 nmol mol^{-1}). In contrast, chronic responses include lesions that develop over days to weeks under lower O_3 concentrations, and accelerated senescence where lesions might not form. Under field conditions, accelerated senescence may be difficult to identify in the absence of a clean air control or other reference point (e.g. O_3-tolerant cultivar) because the cumulative effects on foliar senescence at the end of the season are separated in time from the O_3 events causing the effects. The timing of O_3 episodes in relation to plant development can also have a major effect on the outcome as in the case of soybean in which O_3 exposure during reproductive growth has a much greater impact on yield compared with exposure during vegetative growth (Heagle *et al*. 1991; Miller *et al*. 1991; Morgan *et al*. 2003). The distinction between acute and chronic responses is further complicated by species and genotype differences that lead to a range of effects for a given combination of O_3 concentration and environmental conditions.

Genetic variation in O_3 responses and potential O_3 tolerance mechanisms

Studies of O_3 effects on plants often identify genetic variation as a contributing factor in the observed response. Ozone-sensitive and tolerant cultivars or clones have been reported for many plant species (Wellburn & Wellburn 1996) including crops such as soybean (Tingey, Reinert & Carter 1972; Heagle & Letchworth 1982; Robinson & Britz 2000), snap bean (*Phaseolus vulgaris* L.) (Guzy & Heath 1993), tomato (*Lycopersicon esculentum* Miller) (Temple 1990), clover (*Trifolium repens* L.) (Heagle *et al*. 1993), wheat (Heagle, Miller & Pursley 2000), and potato (*Solanum tuberosum* L.) (Heagle, Miller & Pursley 2003), and natural vegetation such as black cherry (*Prunus serotina* Ehrh.) (Lee *et al*. 2002) and *Plantago* spp. (Wolff, Morgan-Richards & Davison 2000). Studies have also shown that O_3 tolerance is a heritable trait (Damicone & Manning 1987; Reinert & Eason 2000). Although much effort has been made to identify the physiological and biochemical elements of O_3 tolerance, no clear picture has emerged. Based on the oxidative stress model, formation of ROS and other oxidation products is the underlying process involved in the generation and propagation of toxic compounds and abiotic elicitors in plants. Therefore, features of antioxidant metabolism that scavenge or affect the perception of O_3-derived ROS could contribute to O_3 tolerance.

Plants have evolved elaborate systems to combat general oxidative stress based on enzymes that utilize ascorbic acid and glutathione (Noctor & Foyer 1998; see also Foyer & Noctor 2005; this issue), and these antioxidant systems have been linked to O_3 stress tolerance (Runeckles & Chevone 1992; Smirnoff 1996; Chernikova *et al*. 2000; Conklin & Barth 2004). A critical role for ascorbic acid is demonstrated with the *vtc1* mutant of *Arabidopsis* in which a significant decrease in ascorbate content was associated with increased O_3 sensitivity (Conklin, Williams & Last 1996). A more detailed analysis of *vtc1* suggested that in addition to acting as an antioxidant, ascorbic acid serves a regulatory function in the signalling networks that control plant defence responses and leaf senescence (Conklin & Barth 2004). Although it is clear that minimum levels of ascorbic acid and glutathione are required for normal leaf function, the concentrations of these antioxidant metabolites that naturally occur in leaf tissue are not always well correlated with O_3 tolerance (Guzy & Heath 1993; Wellburn & Wellburn 1996; Burkey *et al*. 2000), suggesting a more complex mechanism that remains to be elucidated.

Recent studies have found higher levels of ascorbic acid in the leaf apoplast of certain O_3-tolerant snap bean genotypes compared with sensitive lines (Burkey, Eason & Fiscus 2003), suggesting that localization of ascorbic acid in the cell wall where initial O_3 reactions occur might be important. Leaf extracellular ascorbate content and redox status are affected by O_3 treatment (Castillo & Greppin 1988; Luwe & Heber 1995; Burkey 1999), evidence that extracellular ascorbic acid is involved in detoxification of O_3 and related ROS. Ascorbic acid is initially synthesized inside cells (Smirnoff, Conklin & Loewus 2001) followed by transport between the cytoplasm and extracellular space via specific carriers located in the plasma membrane (Horemans, Foyer & Asard 2000). A model developed by Chameides (1989) and expanded by others (Plöchl *et al*. 2000; Turcsanyi *et al*. 2000) features ascorbic acid in the leaf apoplast as a critical factor in the detoxification of extracellular O_3 and ROS, protecting plasma membranes from oxidative

damage and preventing O_3 injury. Ascorbic acid potentially could serve as either a direct chemical scavenger of O_3, although this mechanism has been questioned (Jakob & Heber 1998), or as a substrate for extracellular enzymes (e.g. ascorbate peroxidase) that attenuate ROS levels and thus affect the propagation of the initial O_3 signal. However, leaf apoplast ascorbic acid levels can be quite low in certain plant species [summarized in Burkey *et al*. (2003)], suggesting that extracellular scavenging of O_3 and ROS by ascorbate may not be a significant factor in all plants. Other compounds with antioxidant properties also exist in the leaf apoplast. Identification of these compounds and their impact on ROS formation and propagation are subjects for future research on O_3 tolerance mechanisms.

Another approach to understanding O_3 responses in plants involves investigations of the potential interaction between chemical signals and specific receptors in the plasma membrane. Booker *et al*. (2004) showed that the epinasty frequently observed for wild-type *Arabidopsis* leaves after O_3 treatment did not occur in *gpa1* mutant plants lacking the alpha subunit of the heterotrimeric G protein complex, suggesting that G-proteins may be involved in the transduction of O_3-derived signals. The binding of O_3 or more likely one of the postulated O_3 reaction products to a G-protein receptor could activate the protein and initiate signal cascade processes that activate target proteins in this signal transduction system. However, further studies will be required to define the possible role G-proteins have in O_3-induced signal transduction and propagation. It is also possible that G-proteins modulate O_3 responses via hormone signal transduction processes. In either case, elements of G-protein pathways involved in O_3 response represent potential molecular targets that might be manipulated to enhance O_3 tolerance although this is speculative at present. The challenge will be to identify targets unique to O_3 so that other important stress responses remain intact (e.g. plant defence responses against pathogen attack). Finally, it is likely that any protective mechanism will come with some metabolic cost that will require evaluation to determine its net benefits.

IMPACTS ON PHOTOSYNTHESIS

Net assimilation

Other than crop yield and visible injury, photosynthesis has been the most broadly studied aspect of plant responses to O_3. Generally, O_3 exposure results in decreased photosynthetic carbon assimilation (Miller 1987b; Runeckles & Chevone 1992; Pell *et al*. 1997; Long & Naidu 2002; Morgan *et al*. 2003). In many cases loss of assimilation capacity was shown to be due primarily to reduced carboxylation efficiency directly related to loss of Rubisco activity. These losses of activity are thought to be due to decreases in Rubisco concentration in the leaves rather than a decrease in activation state. Messenger RNA transcripts for the Rubisco small subunit (*rbc*S) have been noted to decline with O_3 treatment in potato leaves (Glick *et al*. 1995), suggesting that protein synthesis might be inhibited, a notion consistent with observations that Rubisco content in poplar (*Populus* spp.) leaves treated with O_3 does not rise as high as controls during leaf expansion (Pell *et al*. 1997). However, Eckardt & Pell (1994), under conditions where additional synthesis should be minimal, showed a substantial decline in Rubisco content suggesting that the primary cause of the decline in Rubisco due to O_3 exposure was enhanced degradation rather than reduced production.

Because photosynthetic measurements are typically made on the most recently fully expanded upper canopy leaves, in determinate plants, including many soybean lines, it is often difficult to demonstrate differences in net photosynthesis (A_n) due to O_3 until near or after the time when production of new upper canopy leaves has ceased. Then, during most of the reproductive stages, A_n decreased earlier than in control plants (accelerated senescence). This suggests that during vegetative growth, either Rubisco synthesis in newly formed upper canopy leaves is not substantially affected or that these leaves have not accumulated sufficient damage to elicit a response. Vozzo *et al*. (1995) demonstrated significant reductions in A_n on somewhat older soybean leaves that were relatively unaffected by shading but had received sufficient exposure to exhibit visible symptoms, thus lending support to this idea. In contrast Morgan *et al*. (2004), using an indeterminate line were unable to demonstrate any differences due to dose accumulation in a cohort of soybean leaves that developed during vegetative growth. However they did observe decreased A_n in another cohort that developed during reproductive growth and remained near the top of the canopy. These results could well have been due to differences in exposure methodologies, particularly the greater atmospheric coupling to the lower canopy in OTCs as compared to FACE systems. In further contrast to the above observations additional review of the data in two earlier soybean studies (Reid & Fiscus 1998; Reid *et al*. 1998), illustrated in Fig. 2, shows that Rubisco content and activity, chlorophyll content, and A_n were reduced by O_3 early in the vegetative stage and that all but A_n had reached or exceeded clean air levels by early flowering. Afterwards, while chlorophyll content, A_n, and Rubisco activity remained level or declined slightly, Rubisco content continued to rise in the O_3 treatment. Finally in early seed development all these parameters began to decline more precipitously. Throughout this whole experiment chlorophyll content and Rubisco activity tracked each other very well. This suggests that from early flowering through most of seed development O_3 stimulated Rubisco content on a leaf area basis. In addition the question of the role of accelerated senescence, especially during reproductive growth, is one deserving more attention. For example, in Fig. 2 the O_3-induced decline in A_n during reproduction may be due to the normal process of senescence that has been accelerated by O_3 exposure or it may be due simply to the loss of photosynthetically active leaf area due to accumulated O_3 damage. Thus, especially in areas associated with visible lesions, one would measure a mixture of live and dead, or dying, cells and it seems rele-

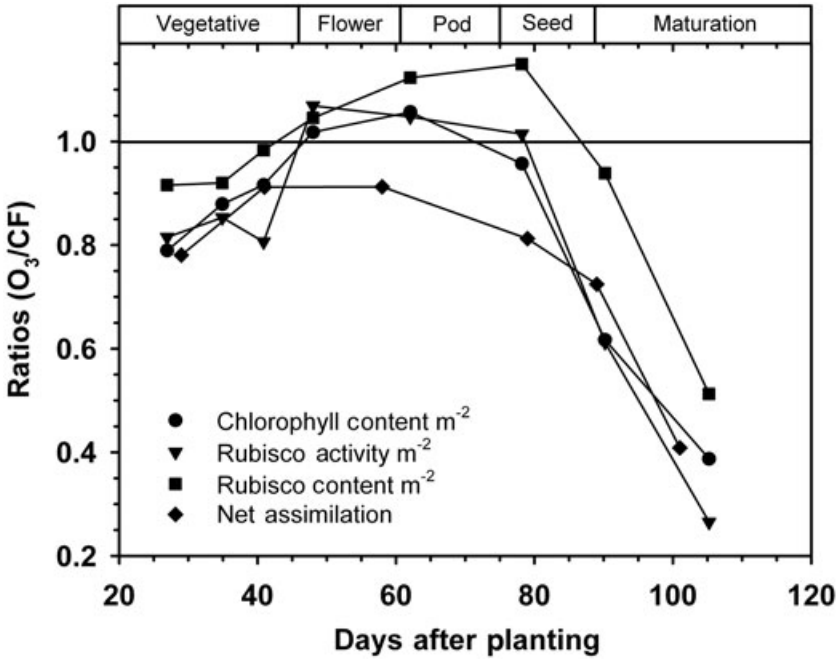

Figure 2. Rubisco quantity and activity, A_n, and chlorophyll content ratios for soybean (cv. Essex) leaves treated with charcoal-filtered air (CF) 25 nmol O_3 mol^{-1} and 1.5 × ambient O_3 (75 nmol mol^{-1}, 12 h average) in open-top field chambers in 1994. The ratios represent the response to O_3 and are calculated as the quantity present in the O_3 treatment compared with the control (O_3/CF). Developmental stages are given in the bar above the graph. Data are from Reid *et al.* (1998) and Reid & Fiscus (1998).

vant to know whether the remaining live cells are functioning at or near normal levels for their age. This possibility is suggested by the fact that from beginning of pod fill through seed maturation when A_n per unit area is declining in this study, A_n per unit Rubisco remained high at 91% of the CF control (difference not significant and data not shown). Further information is necessary to clarify this. Another point deserving attention and bearing on the exposure accumulation comments earlier in the paragraph is the substantially reduced A_n early in the vegetative period. This may have been caused by some inherent sensitivity of these leaves or be the result of enhanced atmospheric coupling due to the more open nature of the canopy at this growth stage.

Photosynthetic electron transport

Detrimental effects of O_3 on photosynthetic electron transport has received increased attention in recent years, and while earlier it had been difficult to demonstrate significant declines in function, evidence is accumulating that in some plants there are demonstrable effects, both on PSII function as well as the xanthophyll cycle components. For instance, significant and substantial declines in F_v/F_m, which represents the efficiency of excitation energy capture by PSII in dark-adapted leaves, have been reported for pumpkin (*Cucurbita pepo* L.) (Ciompi *et al.* 1997; Castagna *et al.* 2001), wheat (*T. durum* and *T. aestivum*) (Reichenauer *et al.* 1998), bean (*P. vulgaris* L.) (Guidi, Di Cagno & Soladatini 2000a; Guidi, Tonini & Soladatini 2000b; Leipner, Oxborough & Baker 2001; Guidi, Degl'Innocenti & Soldatini 2002), tomato (Calatayud & Barreno 2001), and *Festuca pratensis* and turnip (*Brassica napus* L.) (Plazek, Rapacz & Skoczowski 2000). Of these particular studies, three demonstrated an increase in F_o which suggested damaged or deactivated PSII centers (Guidi *et al.* 2000a; Leipner *et al.* 2001; Guidi *et al.* 2002), all occurring in bean. In addition, light saturated photosynthesis (A_{SAT}) decreased in bean (Guidi *et al.* 2000a; Guidi *et al.* 2002), pumpkin (Ciompi *et al.* 1997; Castagna *et al.* 2001), and wheat (Reichenauer *et al.* 1998), although in the latter the effect was confined to older leaves in two of the three lines and was partially reversible in some lines and fully reversible overnight in one. It was unknown whether reversibility was due to a night-time epoxidation of the xanthophyll cycle decreasing subsequent thermal quenching of PSII or to overnight repair of damaged PSII centres. This latter point becomes relevant since a decrease in F_v/F_m may be caused by both a relatively fast reversible down-regulation or a reversible destruction (Osmond 1994). However, the two processes might be distinguished by monitoring effects on F_o in that thermal dissipation by the xanthophyll cycle would lower F_o whereas damage to PSII would increase F_o as was found in bean (Guidi *et al.* 2000a, 2002; Leipner *et al.* 2001). EPR measurements also showed increases in free radicals which Reichenauer *et al.* (1998) suggested might contribute to damage of chloroplast membranes in the sensitive wheat lines. Runeckles & Vaartnou (1997) also observed light-dependent EPR signals in bluegrass (*Poa pratensis* L.), ryegrass (*Lolium perenne* L.), and radish (*Raphanus sativa* L.), consistent with superoxide anion characteristics, on exposure to O_3. The light dependence suggested that the signal was localized in the chloroplast and significantly its appearance was reduced in leaves with increased levels of apoplastic ascorbic acid.

High light is another factor that might interact with O_3 in such a way as to increase or aggravate the effects of O_3 alone through the process of photoinhibition with a result-

ant increase in free radicals in the chloroplast (Massman 2004). Guidi *et al*. (2002) demonstrated that both high light and O_3 decreased F_v/F_m and increased F_o. Furthermore the combined effects were additive. Farage *et al*. (1991), upon exposing wheat leaves to high $[O_3]$ (200 and 400 nmol mol^{-1}), could detect no difference in atrazine binding in isolated thylakoids nor any difference in D1 protein as indicated by western blots using D1-specific antibodies. However, Godde & Buchhold (1992) found that the turnover rate for D1 was increased by exposure of spruce needles to O_3, suggesting both increased degradation and synthesis. In addition, Ciompi *et al*. (1997) found that in young leaves of pumpkin engagement of the xanthophyll cycle appeared to counteract the effects of O_3 on the PSII reaction center preventing a decline in D1 content. However, in mature leaves, even though there was activation of the xanthophyll cycle, the effect appeared inadequate to prevent photoinhibition and loss of D1 protein. The authors suggested that this effect in mature leaves may have been due to the normal decline in antioxidant activity in older leaves. Clearly high light has the potential to induce photoinhibition which could be already sensitized by O_3 exposure in field crops, or inversely, and is an area that requires further investigation, especially under more realistic field conditions.

Direct effects on stomata

Another possible point of attack by O_3 that cannot be ignored is the guard cells. Although much of the evidence to date indicates that reduced g_s is the result and not the cause of reduced assimilation (McKee *et al*. 1995; Fiscus *et al*. 1997; Long & Naidu 2002), as suggested and deduced on the basis of water use efficiency studies (Reich, Schoettle & Amundsen 1985), there is some intriguing work being done on possible direct effects of O_3 on stomatal response which links oxidative stress, in the form of H_2O_2, Ca^{2+} homeostasis of guard cells, and ABA response. This work was recently reviewed by McAinsh *et al*. (2002) while the review by Schroeder *et al*. (2001) provides a more detailed context for these studies. The studies showed that H_2O_2 stimulated increased whole plant cytosolic free Ca ions $[Ca^{2+}]_{cyt}$. The stimulation was highly dose dependent with biphasic kinetics similar to the Ca signature induced by exposure to O_3, which was also highly dose dependent. Using various techniques, increased $[Ca^{2+}]_{cyt}$ was also demonstrated in guard cells in response to both O_3 and H_2O_2. In addition, Pei *et al*. (2000) demonstrated H_2O_2-induced stomatal closure and that ABA induces H_2O_2 production in guard cells and activates plasma membrane Ca-permeable channels. All of these responses were disrupted in the ABA-insensitive *Arabidopsis* mutant *gca2*. Taken together, these and other studies reviewed (McAinsh *et al*. 2002) raise the possibility that O_3 might directly affect stomata through the action of H_2O_2, formed when O_3 enters solution, on the Ca-permeable channels in the guard cell plasma membranes.

O_3–CO_2 interactions on biomass and yield

Along with O_3, atmospheric CO_2 concentrations are also rising, and numerous studies indicate that elevated CO_2 might enhance the productivity of current cropping systems. Long *et al*. (2004) have shown that the magnitude of the response to elevated CO_2 is highly variable, not only across but within species and cultivars. Although there appears to be a genetic component to this variability, some of it may be due to environmental stresses that occurred during plant growth. Ozone is one such possible stress, and it would not be surprising that there are significant physiological interactions between elevated CO_2 and O_3. The most relevant interaction for present purposes is the reduction in g_s at elevated levels of CO_2 that has the effect of preventing reductions of A_n, growth, and yield in many crops grown in the presence of toxic levels of O_3 (Fiscus *et al*. 1997; Olszyk *et al*. 2000; Fiscus *et al*. 2002; Morgan *et al*. 2003; Booker *et al*. 2005). Figure 3 illustrates this interaction in soybean grown in both elevated CO_2 and O_3 (compare with A_n ratio in Fig. 2), and although it is expected that the assimilation ratios in elevated CO_2 are substantially higher than the control, it is interesting to note that elevated CO_2 did not seem to prevent the accelerated senescence characteristic of an O_3 fumigation at current levels of CO_2. Analysis of compiled shoot biomass and yield data (Fiscus *et al*. 2002) show that increased A_n does get translated into an average 30% increase in biomass but often not into yield in clean (charcoal-filtered) air (Fig. 4) (Fiscus *et al*. 2001). The variability in both biomass and yield is large with yields in doubled CO_2 ranging from –37% to +41% in CF air with a mean of +6% across the crops studied. However, in increasing O_3 concentrations both the apparent biomass and yield due to elevated CO_2 (CO_2 fertilization effect) increased dramatically but the variability of the responses remained large. Of course in elevated CO_2, g_s will be reduced, typically by 30% in these studies, so O_3 flux into the leaves and the effective exposure is much reduced. Thus the apparent dramatic yield increases in some non-filtered air experiments might be caused, in part, by the fact that yields are already suppressed by 5–15% by O_3 in ambient air control plants, and the best that elevated CO_2 seemed to do in these experiments was to restore yields to the clean air levels. The wide range of variability in these experiments suggests that in some, CO_2 was not the limiting resource during reproduction. In fact it seems possible, given the large increases in vegetative biomass, that early resource depletion under elevated CO_2 actually may have reduced yield. One would expect that any environmental stress that affects g_s (e.g. drought) would also influence the direct effects of O_3 and lead to large apparent CO_2-stimulated increases in growth and yield. The cause of the distinct variability shown in these experiments is an area that deserves further research.

Impacts on translocation and partitioning

Early in the season chronic exposure to O_3 can inhibit A_n (Fig. 2) and plant growth (Fig. 5), but often the decline in

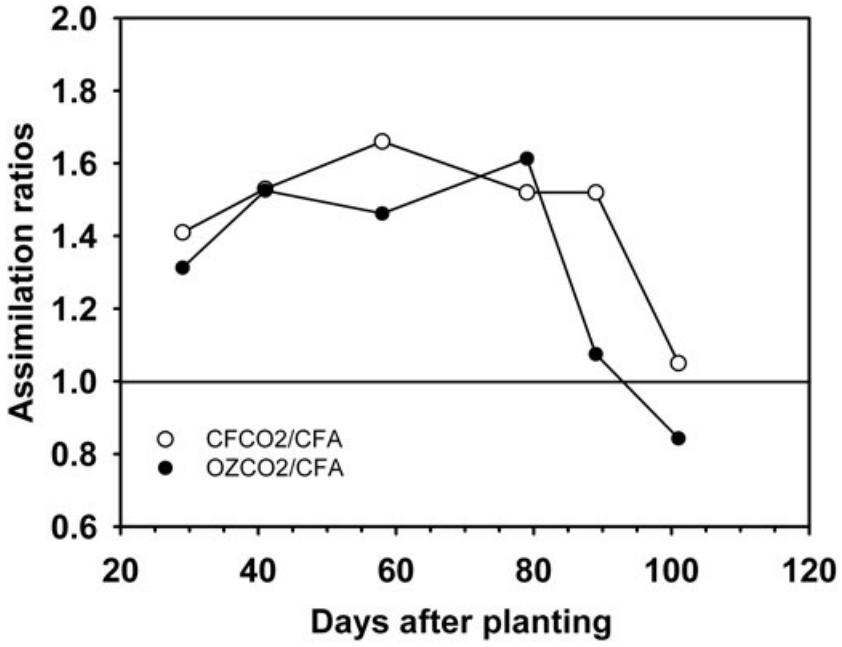

Figure 3. Photosynthetic assimilation ratios for the same 1994 experiment shown in Fig. 2. The ratios are assimilation rates at elevated CO_2 [in the presence (OZCO2) or absence (CFCO2) of elevated O_3] divided by the rates at current ambient CO_2 (CFA). Plants were treated with ambient and twice-ambient concentrations of CO_2 in reciprocal combination with charcoal-filtered air (CF) and 1.5 × ambient O_3 (OZ) in open-top field chambers.

A_n is less than the loss in productivity (Heath & Taylor 1997). For example, Fig. 2 shows that even though the trends of the assimilation ratio never reach or exceed 1, from the late vegetative stage through early seed fill the decrease relative to charcoal-filtered air is in the range where it is difficult to show statistical significance. and although the decrease in the seasonal integral of the A_n ratio (the area between the curve and 1) was less than half the reported percentage decrease in yield, these measurements represent only a small sampling of upper canopy leaves. Typically gas-exchange measurements are made on recently expanded upper canopy leaves in an attempt to sample fully illuminated leaves of the same age and exposure and cannot account for whatever photosynthesis might still be occurring lower in the canopy. However owing to the determinate growth habit of the soybean used in Fig. 2 very few new upper canopy leaves were added after about mid to late pod development. Thus up until about 70 d after planting the leaves sampled received less O_3 exposure while after that the sampled leaves were exposed for increasing periods and that is when the effects became significant. Therefore, the inhibitory effects of O_3 predominately appear during reproductive rather than vegetative growth in crops such as soybean and wheat, which coincide with

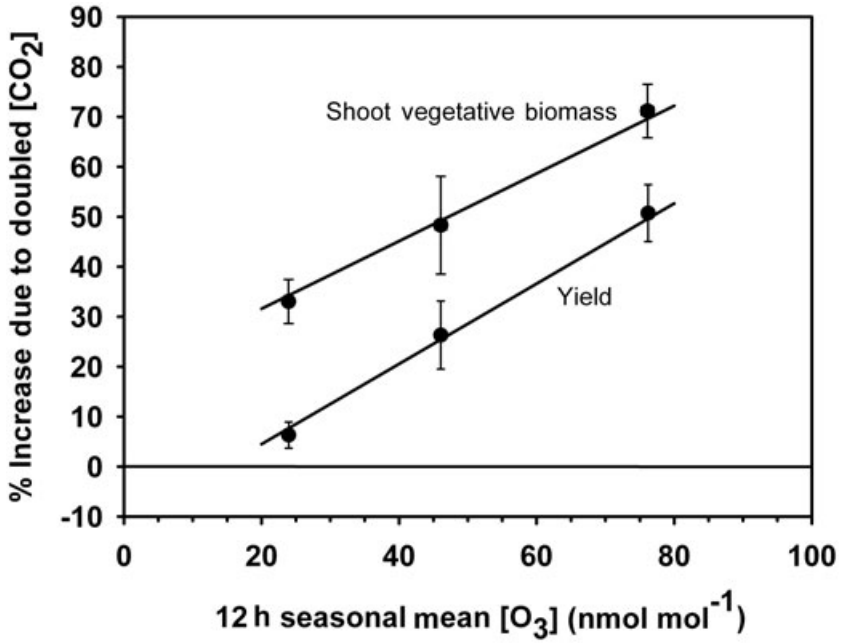

Figure 4. Combined biomass and yield response in twice-ambient concentrations of atmospheric CO_2. Yield data consist of 31 cultivar years of open-top field chamber studies using cotton, rice, soybean, and wheat compiled in Fiscus *et al*. (2002) plus an additional 13 cultivar years for soybean (Booker & Fiscus, unpublished), peanut (Booker & Burkey, unpublished), snap bean (Heagle *et al*. 2002), and potato (Heagle *et al*. 2003). Treatments were either ambient or twice-ambient [CO_2] combined with charcoal-filtered air (CF), non-filtered air (NF), and either CF plus O_3 or NF plus O_3. Increasing O_3 treatment means correspond to CF, NF and +O_3 treatments. Bars are standard errors.

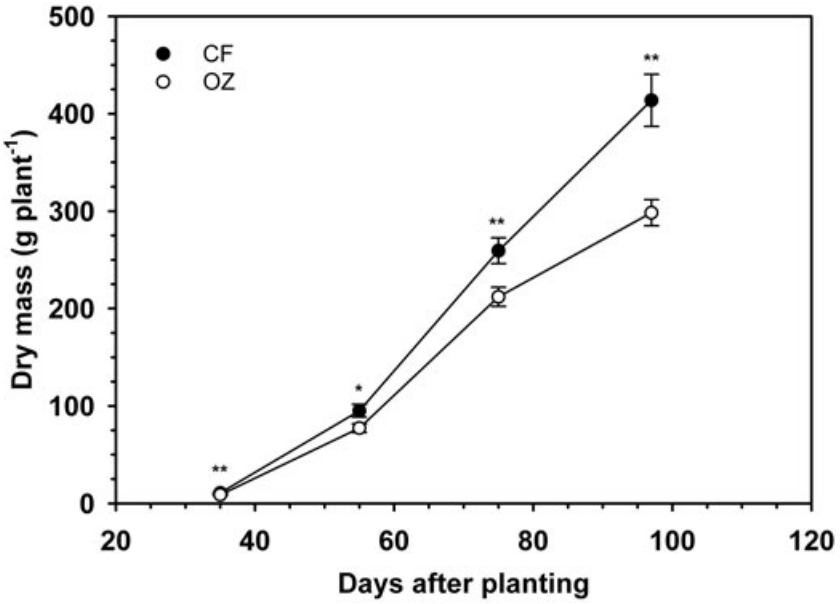

Figure 5. Dry mass plant^{-1} of soybean (cv. Essex) treated from emergence to harvest maturity with charcoal-filtered air (30 nmol O_3 mol^{-1}) (CF) and 1.5 × ambient O_3 levels (74 nmol mol^{-1}) (12 h average) (Oz) in open-top field chambers at Raleigh, NC, USA. Plants were grown in 21 L pots. There were three replicate chambers for each treatment in each year of the 3-year experiment (Booker & Fiscus, unpublished). Values are means ± SE. Significant treatment effects are indicated as $P = 0.05$ (*) and $P = 0.01$ (**).

suppressed A_n and, along with less leaf area, probably comprise the main causes of lower yields (Heagle 1989; Heagle *et al*. 1991; Miller *et al*. 1991; Black *et al*. 2000; Morgan *et al*. 2003). There is a need for more canopy-level studies of photosynthetic processes as affected by O_3. In addition, direct detrimental effects of O_3 on reproductive processes such as pollen germination and tube growth, fertilization, and abscission of flowers, pods, and seeds possibly contribute to suppressed yield as well (Runeckles & Chevone 1992; McKee, Bullimore & Long 1997; Black *et al*. 2000).

Elevated O_3 also lowers the root biomass ratio (fraction of total biomass in root tissue) in numerous crops (Cooley & Manning 1987; Miller 1987a; Runeckles & Chevone 1992; Grantz & Yang 2000). While a meta-analysis of O_3 effects on soybean indicated that inhibitory effects on root and shoot biomass were about equal (Morgan *et al*. 2003), shifts in allocation away from roots toward aerial biomass has been observed in chronic O_3 studies with soybean (Miller, Heagle & Pursley 1998), cotton (Miller *et al*. 1988; Grantz & Yang 2000), and a number of other crops (Cooley & Manning 1987). Ozone injury to lower leaves, which act as the main source of photosynthates for root growth, might explain decreases in root dry mass (Cooley & Manning 1987; Andersen 2003). However, the allometric shift in root biomass ratio in O_3-treated Pima cotton could not be reproduced by pruning canopy or lower-stem leaves to mimic the suppressive effect of O_3 on leaf area (Grantz & Yang 2000). This suggests that inhibitory effects of O_3 on phloem loading, with consequent inhibition of translocation to roots, might be part of the reason why O_3 induces changes in biomass partitioning (Grantz & Farrar 2000; Grantz & Yang 2000). Isotope studies with photoassimilated ^{14}C- and ^{13}C-labelled CO_2 suggested a greater retention of labelled C in leaves and shoots at the expense of translocation to roots (Miller 1987a; Grantz & Farrar 2000; Grantz & Yang 2000). McLaughlin & McConathy (1983) suggested three ways that O_3 might alter translocation: (a) malfunction of the phloem loading process; (b) increased allocation to leaf injury repair; and (c) an altered balance between the leaf and sinks caused by reduced carbon fixation and a greater demand for assimilate in the leaf.

Reduced carbon flow to roots would be expected to negatively affect mycorrhizal development and rhizobial nodulation, which would have feedback effects on mineral nutrient availability for plant growth (Runeckles & Chevone 1992; Fuhrer & Booker 2003). Pathogen susceptibility of roots might be increased as well (Cooley & Manning 1987). Experimental data and modelling techniques further indicated that reduced biomass allocation to roots lowers soil moisture availability that may mediate a decline in g_s, which in turn could reduce canopy-scale water fluxes and O_3 deposition (Grantz, Zhang & Carlson 1999)

CONCLUSION

Regardless of the fine points of timing and proximate cause, the evidence seems clear that in many plants, the loss of economic yield is a direct result of loss of photosynthetic capacity, especially during the reproductive stages of growth. A decrease in leaf area production contributes to suppressed yield as well. Whether there are O_3-induced signals, effects via plant hormones, or metabolic costs of detoxification and repair that impair plant growth from early in their development onwards has yet to be ascertained, but these responses seem plausible.

Owing to the inherent spatial and temporal variability of tropospheric [O_3] it is difficult to generalize about whether chronic or acute exposures in the field impact productivity more. Under any particular set of seasonal circumstances it may be one or the other or neither. Therefore exposure methodologies differ widely and the range of exposures varies from relatively mild chronic exposures lasting several

days, weeks or the entire growing season to acute exposures lasting from a few hours to a few days. Experimental objectives will determine the preferred approach and when assessing productivity losses in crops or natural systems it may even be desirable to combine the two, overlaying acute exposures at developmental periods suspected of being particularly sensitive. But, acute exposures alone may be more revealing for uncovering damage mechanisms, especially those suspected of being on the front line of defence. However, acute exposures, especially to plants that have grown in relatively O_3-free air, may be difficult to interpret. For example, a short, high O_3 exposure may overwhelm plant defences and disrupt membrane lipids and proteins causing extensive leakage of cellular components and uncontrolled cell or tissue death, which rarely occurs in the current environment although similar responses might occur on a micro-scale in substomatal cells and appear as a hypersensitive response to pathogens. The choice of O_3 treatment methodology depends on the goal of the research, whether that is to determine the effects of current ambient O_3 concentrations on yield [e.g. NCLAN, in which open top chambers were used to treat plants with a range of O_3 concentrations that encompassed ambient air concentrations (Heagle 1989)], or to predict effects of future levels of ambient O_3 compared with current levels [e.g. SoyFACE, in which plants are treated with ambient air and ambient air plus O_3 in a free air system (http://www.soyface.uiuc.edu/index.htm)], or to investigate mechanisms of O_3 damage with the aim of improving crop tolerance to O_3 stress (e.g. identification of genetic traits controlling sensitivity to O_3). Each approach has its advantages and drawbacks, and choices depend on objectives and economic feasibility.

However, there is unmistakable evidence that increasing concentrations of atmospheric CO_2 will ameliorate O_3 damage to a number of crops and woody plants. This interaction will likely continue in the foreseeable future as atmospheric CO_2-enrichment and emission of tropospheric O_3 precursors continue to increase. While this might be seen as a fortunate coincidence at present, it is difficult to predict how interactions between these factors and other possible changes in global climate will play out. At present, it is reasonable to conclude that ambient O_3 detracts from the total crop productivity possible in clean air in many regions of the world and thus may diminish the quantity of carbon eventually sequestered in soils. Ozone flux models will likely require modification to account for the influence of elevated CO_2 on g_s and possibly metabolic changes that affect injury threshold levels. Further, O_3 flux models have the potential to help identify key physiological and biochemical processes in the network of plant responses to O_3. Advances in our ability to genetically dissect mechanisms involved in O_3 toxicity should lead to better understanding of differential sensitivity to O_3, and oxidative stress in general, among crop species and cultivars with the goal of developing crops with improved performance in a changing global climate. This research also will aid in better assessments of the costs in terms of potential world-wide food production associated with current and future levels of tropospheric O_3 pollution that underpin governmental air quality regulations.

REFERENCES

Adams R.M. & Horst R.L. Jr (2003) Future directions in air quality research: economic issues. *Environment International* **29,** 289–302.

Andersen C. (2003) Source-sink balance and carbon allocation below ground in plants exposed to ozone. *New Phytologist* **157,** 213–228.

Ashmore M. (2005) Assessing the future global impacts of ozone on vegetation. *Plant, Cell and Environment* **28**, doi:10.1111/j.1365-3040.2005.01341.x

Black V.J., Black C.R., Roberts J.A. & Stewart C.A. (2000) Impact of ozone on the reproductive development of plants. *New Phytologist* **147,** 421–447.

Booker F.L., Burkey K.O., Overmyer K. & Jones A.M. (2004) Differential responses of G-protein *Arabidopsis thaliana* mutants to ozone. *New Phytologist* **162,** 633–641.

Booker F.L., Fiscus E.L. & Miller J.E. (2004) Combined effects of elevated atmospheric carbon dioxide and ozone on soybean whole-plant water use. *Environmental Management* **33,** S355–S362.

Booker F.L., Miller J.E., Fiscus E.L., Pursley W.A. & Stefanski L.A. (2005) Comparative responses of container- versus ground-grown soybean to elevated CO_2 and O_3. *Crop Science* **45**, 883–895.

Burkey K.O. (1999) Effects of ozone on apoplast/cytoplasm partitioning of ascorbic acid in snap bean. *Physiologia Plantarum* **107,** 188–193.

Burkey K.O., Eason G. & Fiscus E.L. (2003) Factors that affect leaf extracellular ascorbic acid content and redox status. *Physiologia Plantarum* **117,** 51–57.

Burkey K.O., Wei C., Palmer G., Ghosh P. & Fenner G.P. (2000) Antioxidant metabolite levels in ozone-sensitive and tolerant genotypes of snap bean. *Physiologia Plantarum* **110,** 195–200.

Calatayud A. & Barreno E. (2001) Chlorophyll *a* fluorescence, antioxidant enzymes and lipid peroxidation in tomato in response to ozone and benomyl. *Environmental Pollution* **115,** 283–289.

Castagna A., Nali C., Ciompi S., Lorenzini G., Soldatini G.F. & Ranieri A. (2001) Ozone exposure affects photosynthesis of pumpkin (*Cucurbita pepo*) plants. *New Phytologist* **152,** 223–229.

Castillo F.J. & Greppin H. (1988) Extracellular ascorbic acid and enzyme activities related to ascorbic acid metabolism in *Sedum album* L. leaves after ozone exposure. *Environmental and Experimental Botany* **28,** 231–2387.

Castillo F.J. & Heath R.L. (1990) Ca^{2+} transport in membrane vesicles from pinto bean leaves and its alteration after ozone exposure. *Plant Physiology* **94,** 788–795.

Chameides W.L. (1989) The chemistry of ozone deposition to plant leaves: role of ascorbic acid. *Environmental Science and Technology* **23,** 595–600.

Chernikova T., Robinson J.M., Lee E.H. & Mulchi C.L. (2000) Ozone tolerance and antioxidant enzyme activity in soybean cultivars. *Photosynthesis Research* **64,** 15–26.

Ciompi S., Castagna A., Ranieri A., Nali C., Lorenzini G. & Soldatini F.G. (1997) CO_2 assimilation, xanthophyll cycle pigments and PSII efficiency in pumpkin plants as affected by ozone fumigation. *Physiologia Plantarum* **101,** 881–889.

Conklin P.L. & Barth C. (2004) Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. *Plant, Cell and Environment* **27,** 959–970.

Conklin P.L., Williams E.H. & Last R.L. (1996) Environmental stress sensitivity of an ascorbic acid-deficient *Arabidopsis* mutant. *Proceedings of the National Academy of Sciences of the USA* **93,** 9970–9974.

Cooley D.R. & Manning W.J. (1987) The impact of ozone on assimilate partitioning in plants: a review. *Environmental Pollution* **47,** 95–113.

Damicone J.P. & Manning W.J. (1987) Foliar sensitivity of soybeans from early maturity groups to ozone and inheritance of injury response. *Plant Disease* **71,** 332–336.

Dominy P.J. & Heath R.L. (1985) Inhibition of the K^+-stimulated ATPase of the plasmalemma of pinto bean leaves by ozone. *Plant Physiology* **77,** 43–45.

Eckardt N.A. & Pell E. (1994) O_3-induced degradation of Rubisco protein and loss of Rubisco mRNA in relation to leaf age in *Solanum tuberosum* L. *New Phytologist* **127,** 741–748.

Emberson L.D., Ashmore M.R., Cambridge H.M., Simpson D. & Tuovinen J.P. (2000) Modelling stomatal ozone flux across Europe. *Environmental Pollution* **109,** 403–413.

Farage P.K., Long S.P., Lechner E.G. & Baker N.R. (1991) The sequence of change within the photosynthetic apparatus of wheat following short-term exposure to ozone. *Plant Physiology* **95,** 529–535.

Fiscus E.L., Booker F.L., Reid C.D. & Miller J.E. (2001) Unconsidered environmental stresses may cause overestimates of the CO_2-fertilization effect. *Paper presented at the 12th International Congress on Photosynthesis*, Brisbane, Australia. http://www.publish.csiro.au/paper/SA0403642.htm

Fiscus E.L., Miller J.E., Booker F.L., Heagle A.S. & Reid C.D. (2002) The impact of ozone and other limitations on the crop productivity response to CO_2. *Technology* **8,** 181–192.

Fiscus E.L., Reid C.D., Miller J.E. & Heagle A.S. (1997) Elevated CO_2 reduces O_3 flux and O_3-induced yield losses in soybeans: possible implications for elevated CO_2 studies. *Journal of Experimental Botany* **48,** 307–313.

Foyer C. H. & Noctor G. (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell and Environment* **28,** doi:10.1111/j.1365-3040.2005.01327.x

Fuhrer J. & Booker F.L. (2003) Ecological issues related to ozone: agricultural issues. *Environment International* **29,** 141–154.

Fuhrer J., Skarby L. & Ashmore M. (1997) Critical levels for ozone effects on vegetation in Europe. *Environmental Pollution* **97,** 91–106.

Glick R.E., Schlagnhaufer C.D., Arteca R.N. & Pell E.J. (1995) Ozone-induced ethylene emission accelerates the loss of ribulose-1,5-bisphosphate carboxylase/oxygenase and nuclear-encoded mRNAs in senescing potato leaves. *Plant Physiology* **109,** 891–898.

Godde D. & Buchhold J. (1992) Effect of long term fumigation with ozone on the turnover of the D-1 reaction center polypeptide of photosystem II in spruce (*Picea abies*). *Physiologia Plantarum* **86,** 568–574.

Grantz D.A. & Farrar J.F. (2000) Ozone inhibits phloem loading from a transport pool: Compartmental efflux analysis in Pima cotton. *Australian Journal of Plant Physiology* **27,** 859–868.

Grantz D.A. & Yang S. (2000) Ozone impacts on allometry and root hydraulic conductance are not mediated by source limitation nor developmental age. *Journal of Experimental Botany* **51,** 919–927.

Grantz D.A., Zhang X. & Carlson T. (1999) Observations and model simulations link stomatal inhibition to impaired hydraulic conductance following ozone exposure in cotton. *Plant, Cell and Environment* **22,** 1201–1210.

Grimes H.D., Perkins K.K. & Boss W.F. (1983) Ozone degrades into hydroxyl radical under physiological conditions. *Plant Physiology* **72,** 1016–1020.

Grünhage L., Haenel H.D. & Jäger H.J. (2000) The exchange of ozone between vegetation and atmosphere: micrometeorological measurement techniques and models. *Environmental Pollution* **109,** 373–392.

Guidi L., Degl'Innocenti E. & Soldatini G.F. (2002) Assimilation of CO_2, enzyme activation and photosynthetic electron transport in bean leaves, as affected by high light and ozone. *New Phytologist* **156,** 377–388.

Guidi L., Di Cagno R. & Soladatini G.F. (2000a) Screening of bean cultivars for their response to ozone as evaluated by visible symptoms and leaf chlorophyll fluorescence. *Environmental Pollution* **107,** 349–355.

Guidi L., Tonini M. & Soladatini G.F. (2000b) Effects of high light and ozone fumigation on photosynthesis in *Phaseolus vulgaris*. *Plant Physiology and Biochemistry* **38,** 717–725.

Gunnison A.F. & Hatch G.E. (1999) O_3-induced inflammation in prepregnant, pregnant, and lactating rats correlates with O_3 dose estimated by ^{18}O. *American Journal of Physiology* **276,** L332–L340.

Guzy M.R. & Heath R.L. (1993) Responses 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 of Phytopathology* **27,** 397–423.

Heagle A.S. & Letchworth M.B. (1982) Relationships among injury, growth, and yield responses of soybean cultivars exposed to ozone at different light intensities. *Journal of Environmental Quality* **11,** 690–694.

Heagle A.S., Miller J.E., Booker F.L. & Pursley W.A. (1999) Ozone stress, carbon dioxide enrichment, and nitrogen fertility interactions in cotton. *Crop Science* **39,** 731–741.

Heagle A.S., Miller J.E., Burkey K.O., Eason G. & Pursley W.A. (2002) Growth and yield responses of snap bean to mixtures of carbon dioxide and ozone. *Journal of Environmental Quality* **31,** 2008–2014.

Heagle A.S., Miller J.E. & Pursley W.A. (2000) Growth and yield responses of winter wheat to mixtures of ozone and carbon dioxide. *Crop Science* **40,** 1656–1664.

Heagle A.S., Miller J.E. & Pursley W.A. (2003) Growth and yield responses of potato to mixtures of carbon dioxide and ozone. *Journal of Environmental Quality* **32,** 1603–1610.

Heagle A.S., Miller J.E., Rawlings J.O. & Vozzo S.F. (1991) Effect of growth stage on soybean response to chronic ozone exposure. *Journal of Environmental Quality* **20,** 562–570.

Heagle A.S., Miller J.E., Sherrill D.E. & Rawlings J.O. (1993) Effects of ozone and carbon dioxide mixtures on two clones of white clover. *New Phytologist* **123,** 751–762.

Heath R.L. (1987) The biochemistry of ozone attack on the plasma membrane of plant cells. *Recent Advances in Phytochemistry* **21,** 29–54.

Heath R.L. & Taylor G.E. Jr (1997) Physiological processes and plant responses to ozone exposure. In *Forest Decline and Ozone: a Comparison of Controlled Chamber and Field Experiments* (eds H. Sandermann, A.R. Wellburn & R.L. Heath), pp. 317–368. Springer-Verlag, Berlin, Germany.

Heck W.W., Adams R.M., Cure W.W., Heagle A.S., Heggestad H.E., Kohut R.J., Kress L.W., Rawlings J.O. & Taylor O.C. (1983) A reassessment of crop loss from ozone. *Environmental Science and Technology* **17,** 572A–581A.

Hewitt C.N., Kok G.L. & Rall R. (1990) Hydroperoxides in plants exposed to ozone mediate air pollution damage to alkene emitters. *Nature* **344,** 56–58.

Horemans N., Foyer C.H. & Asard H. (2000) Transport and action of ascorbate at the plasma membrane. *Trends in Plant Science* **5,** 263–267.

Humphries S.W. & Long S.P. (1995) WIMOVAC: a software package for modelling the dynamics of plant leaf and canopy photosynthesis. *CABIOS (Computer Applications in BioSciences)* **11,** 361–371.

Jakob B. & Heber U. (1998) Apoplastic ascorbate does not prevent the oxidation of fluorescent amphiphilic dyes by ambient and elevated concentrations of ozone in leaves. *Plant and Cell Physiology* **39,** 313–322.

Jarvis P.G. (1976) The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. *Philosophical Transactions of the Royal Society of London. Series B, Biology Sciences* **273,** 593–610.

Kangasjärvi J., Talvinen J., Utriainen M. & Karjalainen R. (1994) Plant defence systems induced by ozone. *Plant, Cell and Environment* **17,** 783–794.

Kangasjärvi J., Jaspers P. & Kollist H. (2005) Signalling and cell death in ozone-exposed plants. *Plant, Cell and Environment* **28,** doi:10.1111/j.1365-3040.2005.01325.x

Kirichenko A., Li L., Morandi M.T. & Holian A. (1996) 4-Hydroxy-2-nonenal-protein adducts and apoptosis in murine lung cells after acute ozone exposure. *Toxicology and Applied Pharmacology* **141,** 416–424.

Kuik O.J., Helming J.F.M., Dorland C. & Spaninks F.A. (2000) The economic benefits to agriculture of a reduction of low level ozone pollution in the Netherlands. *European Review of Agricultural Economics* **27,** 75–90.

Lee J.C., Steiner K.C., Zhang J.W. & Skelly J.M. (2002) Heritability of ozone sensitivity in open-pollinated families of black cherry (*Prunus serotina* Ehrh.). *Forest Science* **48,** 111–117.

Lefohn A.S. (1992) The characterization of ambient ozone exposures. In *Surface Level Ozone Exposures and Their Effects on Vegetation* (ed. A.S. Lefohn), pp. 31–92. Lewis Publishers, Inc., Chelsea, MI, USA.

Leipner J., Oxborough K. & Baker N.R. (2001) Primary sites of ozone-induced perturbations of photosynthesis in leaves: identification and characterization in *Phaseolus vulgaris* using high resolution chlorophyll fluorescence imaging. *Journal of Experimental Botany* **52,** 1689–1696.

Long S.P., Ainsworth E.A., Rogers A. & Ort D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology* **55,** 591–628.

Long S.P. & Naidu S.L. (2002) Effects of oxidants at the biochemical, cell and physiological levels, with particular reference to ozone. In *Air Pollution and Plant Life* (eds J.N. B. Bell & M. Treshow), pp. 69–88. John Wiley & Sons, Ltd., West Sussex.

Loreto F. & Velikova V. (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology* **127,** 1781–1787.

Luwe M. & Heber U. (1995) Ozone detoxification in the apoplasm and symplasm of spinach, broad bean and beech leaves at ambient and elevated concentrations of ozone in air. *Planta* **197,** 448–455.

Martin M.J., Farage P.K., Humphries S.W. & Long S.P. (2000) Can the stomatal changes caused by acute ozone exposure be predicted by changes occurring in the mesophyll? A simplification for models of vegetation response to the global increase in tropospheric elevated ozone episodes. *Australian Journal of Plant Physiology* **27,** 211–219.

Massman W.J. (2004) Toward an ozone standard to protect vegetation based on effective dose: a review of deposition resistances and a possible metric. *Atmospheric Environment* **38,** 2323–2337.

Mauzerall D.L. & Wang X.P. (2001) Protecting agricultural crops from the effects of tropospheric ozone exposure: Reconciling science and standard setting in the United States, Europe, and Asia. *Annual Review of Energy and the Environment* **26,** 237–268.

McAinsh M.R., Evans N.H., Montgomery L.T. & North K.A. (2002) Calcium signalling in stomatal responses to pollutants. *New Phytologist* **153,** 441–447.

McKee I.F., Bullimore J.F. & Long S.P. (1997) Will elevated CO_2 concentrations protect the yield of wheat from O_3 damage? *Plant, Cell and Environment* **20,** 77–84.

McKee I.F., Farage P.K. & Long S.P. (1995) The interactive effects of elevated CO_2 and O_3 concentration on photosynthesis in spring wheat. *Photosynthesis Research* **45,** 111–119.

McLaughlin S.B. & McConathy R.K. (1983) Effects of SO_2 and O_3 on allocation of ^{14}C-labeled photosynthate in *Phaseolus vulgaris*. *Plant Physiology* **73,** 630–635.

Mehlhorn H., Tabner B.J. & Wellburn A.R. (1990) Electron spin resonance evidence for the formation of free radicals in plants exposed to ozone. *Physiologia Plantarum* **79,** 377–383.

Miller J.E. (1987a) Effects of ozone and sulfur dioxide stress on growth and carbon allocation in plants. *Recent Advances in Phytochemistry* **21,** 55–100.

Miller J.E. (1987b) Effects on photosynthesis, carbon allocation, and plant growth associated with air pollutant stress. In *Assessment of Crop Loss from Air Pollutants* (eds W.W. Heck, O.C. Taylor & D.T. Tingey), pp. 287–314. Elsevier Applied Science, London, UK.

Miller J.E., Heagle A.S. & Pursley W.A. (1998) Influence of ozone stress on soybean response to carbon dioxide enrichment. II. Biomass and development. *Crop Science* **38,** 122–128.

Miller J.E., Patterson R.P., Heagle A.S., Pursley W.A. & Heck W.W. (1988) Growth of cotton under chronic ozone stress at two levels of soil moisture. *Journal of Environmental Quality* **17,** 635–643.

Miller J.E., Pursley W.A., Vozzo S.F. & Heagle A.S. (1991) Response of net carbon exchange rate of soybean to ozone at different stages of growth and its relation to yield. *Journal of Environmental Quality* **20,** 571–575.

Morgan P.B., Ainsworth E.A. & Long S.P. (2003) How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. *Plant, Cell and Environment* **26,** 1317–1328.

Morgan P.B., Bernacchi C.J., Ort D.R. & Long S.P. (2004) An *in vivo* analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. *Plant Physiology* **135,** 2348–2357.

Murphy J.J., Delucchi M.A., McCubbin D.R. & Kim H.J. (1999) The cost of crop damage caused by ozone air pollution from motor vehicles. *Journal of Environmental Management* **55,** 365–376.

Musselman R.C. & Massman W.J. (1999) Ozone flux to vegetation and its relationship to plant response and ambient air quality standards. *Atmospheric Environment* **33,** 65–73.

Noctor G. & Foyer C.H. (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49,** 249–279.

Olszyk D.M., Tingey D.T., Watrud L., Seidler R. & Andersen C. (2000) Interactive effects of O_3 and CO_2: implications for terrestrial ecosystems. In *Trace Gas Emissions and Plants* (ed. S.N. Singh), pp. 97–136. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Osmond C.B. (1994) What is photoinhibition? Some insights from comparison of shade and sun plants. In *Photoinhibition of Photosynthesis – from Molecular Mechanisms to the Field* (eds N.R. Baker & J.R. Bowyer), pp. 1–24. Bios Scientific Publishers, Oxford, UK.

Overmyer K., Brosche M. & Kangasjärvi J. (2003) Reactive oxygen species and hormonal control of cell death. *Trends in Plant Science* **8,** 335–342.

Overmyer K., Tuominen H., Kettunen R., Betz C., Langebartels C., Sandermann H.J. & Kangasjärvi J. (2000) Ozone-sensitive Arabidopsis *rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. *Plant Cell* **12,** 1849–1862.

Pell E., Schlagnhaufer C.D. & Arteca R.N. (1997) Ozone-induced oxidative stress: mechanisms of action and reaction. *Physiologia Plantarum* **100,** 264–273.

Pendall E., Bridgham S., Hanson P.J., *et al.* (2004) Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. *New Phytologist* **162,** 311–322.

Pei Z.-M., Murata Y., Benning G., Thomine S., Klüsener B., Allen G.J., Grill E. & Schroeder J.I. (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* **406,** 731–734.

Plazek A., Rapacz M. & Skoczowski A. (2000) Effects of ozone fumigation on photosynthesis and membrane permeability in leaves of spring barley, meadow fescue and winter rape. *Photosynthetica* **38,** 409–413.

Pleijel H., Danielsson H., Ojanpera K., Temmerman L.D., Hogy P., Badiani M. & Karlsson P.E. (2004) Relationships between ozone exposure and yield loss in European wheat and potato – a comparison of concentration- and flux-based exposure indices. *Atmospheric Environment* **38,** 2259–2269.

Plöchl M., Lyons T., Ollerenshaw J. & Barnes J. (2000) Simulating ozone detoxification in the leaf apoplast through the direct reaction with ascorbate. *Planta* **210,** 454–467.

Prather M., Ehhalt D., Dentener F., *et al.* (2001) Atmospheric chemistry and greenhouse gases. In *Climate Change 2001: the Scientific Basis Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskell & C.A. Johnson), pp. 239–287. Cambridge University Press, Cambridge, UK.

Pryor W.A. (1994) Mechanisms of radical formation from reactions of ozone with target molecules in the lung. *Free Radical Biology and Medicine* **17,** 451–465.

Rao M.V. & Davis K.R. (2001) The physiology of ozone induced cell death. *Planta* **213,** 682–690.

Rao M.V., Lee H.-I., Creelman R.A., Mullet J.E. & Davis K.R. (2000) Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. *Plant Cell* **12,** 1633–1646.

Rao M.V., Lee H.I. & Davis K.R. (2002) Ozone-induced ethylene production is dependent on salicylic acid, and both salicylic acid and ethylene act in concert to regulate ozone-induced cell death. *Plant Journal* **32,** 447–456.

Reich P.B., Schoettle A.W. & Amundsen R.G. (1985) Effects of low concentrations of O_3, Leaf age and water stress on leaf diffusive conductance and water use efficiency in soybean. *Physiologia Plantarum* **63,** 58–64.

Reichenauer T.G., Goodman B.A., Kostecki P. & Soja G. (1998) Ozone sensitivity in *Triticum durum* and *T. aestivum* with respect to leaf injury, photosynthetic activity and free radical content. *Physiologia Plantarum* **104,** 681–686.

Reid C.D. & Fiscus E.L. (1998) Effects of elevated [CO_2] and/or ozone on limitations to CO_2 assimilation in soybean *(Glycine max)*. *Journal of Experimental Botany* **49,** 885–895.

Reid C.D., Fiscus E.L. & Burkey K.O. (1998) Combined effects of chronic ozone and elevated CO_2 on Rubisco activity and leaf components in soybean (*Glycine max*). *Journal of Experimental Botany* **49,** 1999–2011.

Reid C.D., Fiscus E.L. & Burkey K.O. (1999) Effects of chronic ozone and elevated atmospheric CO_2 concentrations on ribulose-1,5-bisphosphate in soybean (*Glycine max*). *Physiologia Plantarum* **106,** 378–385.

Reinert R.A. & Eason G. (2000) Genetic control of O_3 sensitivity in a cross between two cultivars of snap bean. *Journal of the American Society of Horticultural Science* **125,** 222–227.

Robinson J.M. & Britz S.J. (2000) Tolerance of a field grown soybean cultivar to elevated ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydroascorbate redox status, and long term photosynthetic productivity. *Photosynthesis Research* **64,** 77–87.

Runeckles V.C. (1992) Uptake of ozone by vegetation. In *Surface Level Ozone Exposures and Their Effects on Vegetation* (ed. A.S. Lefohn), pp. 157–188. Lewis Publishers, Inc., Chelsea, MI, USA.

Runeckles V.C. & Chevone B.I. (1992) Crop responses to ozone. In *Surface Level Ozone Exposures and Their Effects on Vegetation* (ed. A.S. Lefohn), pp. 189–270. Lewis Publishers, Inc., Chelsea, MI, USA.

Runeckles V.C. & Vaartnou M. (1997) EPR evidence for superoxide anion formation in leaves during exposure to low levels of ozone. *Plant, Cell and Environment* **20,** 306–314.

Sandermann H. Jr, Ernst D., Heller W. & Langebartels C. (1998) Ozone: an abiotic elicitor of plant defence reactions. *Trends in Plant Science* **3,** 47–50.

Schraudner M., Moeder W., Wiese C., Van Camp W., Inzé D., Langebartels C. & Sandermann H. Jr (1998) Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco Bel W3. *Plant Journal* **16,** 235–245.

Schroeder J.I., Allen G.J., Hugouvieux V., Kwak J.M. & Waner D. (2001) Guard cell signal transduction. *Annual Review of Plant Physiology and Molecular Biology* **52,** 627–658.

Sharkey T.D. & Yeh S. (2001) Isoprene emission from plants. *Annual Review of Plant Physiology and Molecular Biology* **52,** 407–436.

Sharma Y.K., Leon J., Raskin I. & Davis K.R. (1996) Ozone-induced responses in *Arabidopsis thaliana*: The role of salicylic acid in the accumulation of defense-related transcripts and induced resistance. *Proceedings of the National Academy of Sciences of the USA* **93,** 5099–5104.

Smirnoff N. (1996) The function and metabolism of ascorbic acid in plants. *Annals of Botany* **78,** 661–669.

Smirnoff N., Conklin P.L. & Loewus F.A. (2001) Biosynthesis of ascorbic acid in plants: a renaissance. *Annual Review of Plant Physiology and Molecular Biology* **52,** 437–467.

Tamaoki M., Matsuyama T., Kanna M., Nakajima N., Kubo A., Aono M. & Saji J. (2003) Differential ozone sensitivity among *Arabidopsis* accessions and its relevance to ethylene synthesis. *Planta* **216,** 552–560.

Temple P.J. (1990) Growth and yield of processing tomato (*Lycopersicon esculentum* Mill.) cultivars to ozone. *Environmental and Experimental Botany* **30,** 283–291.

Tingey D.T. & Hogsett W.E. (1985) Water stress reduces ozone injury via a stomatal mechanism. *Plant Physiology* **77,** 944–947.

Tingey D.T., Reinert R., A. & Carter H.B. (1972) Soybean cultivars: acute foliar response to ozone. *Crop Science* **12,** 268–270.

Tuovinen J.P. (2000) Assessing vegetation exposure to ozone: properties of the AOT40 index and modifications by deposition modelling. *Environmental Pollution* **109,** 361–372.

Turcsanyi E. Lyons T. Plöchl M. & Barnes J. (2000) Does ascorbate in the mesophyll cell walls form the first line of defence against ozone? Testing the concept using broad bean (*Vicia faba* L.). *Journal of Experimental Botany* **51,** 901–910.

US Environmental Protection Agency. (1996) *Air Quality Criteria for Ozone and Other Photochemical Oxidants*, Vol. III. Report no. EPA/600/P-93/004bF. US Environmental Protection Agency,

Office of Air Quality Planning and Standards, Research Triangle Park, NC, USA.

UNECE (2000) *Review of Strategies and Policies for Air Pollution Abatement.* UNECE Convention on Long–Range Trans Boundary Air Pollution. http://www.unece.org/env/lrtap/conv/conclusi.htm.

UNECE (2004a) *Air Pollution and Vegetation*. ICP Vegetation Annual Report 2003/2004. UNECE ICP Vegetation Coordination Centre, CEH Bangor, UK.

UNECE (2004b) *Manual on Methodologies and Criteria for Modelling and Mapping Critical Loads & Levels and Air Pollution Effects, Risks and Trends*. Umweltbundesamt, Berlin, Germany.

Vahala J., Ruonala R., Keinanen M., Tuominen H. & Kangasjärvi J. (2003) Ethylene insensitivity modulates ozone-induced cell death in birch. *Plant Physiology* **132,** 185–195.

Vozzo S.F., Miller J.E., Pursley W.A. & Heagle A.S. (1995) Effects of ozone and water deficit on field-grown soybean. I. Leaf gas exchange. *Journal of Environmental Quality* **24,** 663–670.

Wellburn F.A.M. & Wellburn A.R. (1996) Variable patterns of antioxidant protection but similar ethene emission differences in several ozone-sensitive and ozone-tolerant plant selections. *Plant, Cell and Environment* **19,** 754–760.

Wohlgemuth H., Mittelstrass K., Kschieschan S., Bender J., Weigel H.-J., Overmyer K., Kangasjärvi J., Sandermann H.J. & Langebartels C. (2002) Activation of an oxidative burst is a general feature of sensitive plants exposed to the air pollutant ozone. *Plant, Cell and Environment* **25,** 717–726.

Wolff K., Morgan-Richards M. & Davison A.W. (2000) Patterns of molecular genetic variation in *Plantago major* and *P. intermedia* in relation to ozone resistance. *New Phytologist* **145,** 501–509.

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