PLANT RESPONSES TO ELEVATED CO₂ AND INTERACTIONS WITH O₃

B. F. T. Rudorff, Charles Lee Mulchi and Edward H. Lee

1National Space Research Institute (INPE), Remote Sensing Division, Caixa Postal 315, 12200-970, São José dos Campos, SP, Brazil.
2Department of Natural Resources Sciences & Landscape Architecture, University of Maryland, College Park, Maryland 20742, USA.
3Climate Stress Laboratory, United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland 20705, USA.

1. Introduction

Plant growth simulation models designated to assess the future impacts of such complete phenomenon as climate change on crop growth and productivity, have typically considered only the beneficial effects of rising atmospheric CO₂ concentration on plants, especially C₄ species. Such models largely neglect potential negative impacts of phytotoxic gases such as O₃ on crops (Adams et al., 1990; Stockle et al., 1992).

Tropospheric CO₂ and O₃ are commonly referred to as "greenhouse" gases because of their abilities to absorb infrared radiation being emitted by Earth resulting in the reemission of this energy into the troposphere (Krupa and Kickert, 1989). Other trace gases of increasing concern include methane (CH₄), nitrous oxide (N₂O), methyl chloride (CH₃Cl) and chlorofluorocarbons (CFC's), because of their potential for disrupting stratospheric O₃ as well as their "greenhouse" properties (Worrest et al., 1989).

Anthropogenic increases in both CO₂ and O₃ are mainly related to the heavy dependence by industrialized nations on fossil fuels as energy sources. Carbon dioxide is produced during the combustion of organic matter. Tropospheric O₃ is the product of photochemical reactions involving oxides of nitrogen (NOₓ) and volatile organic compounds (VOC's) (Krupa and Kickert, 1989; Barnes and Welburn, 1998) and transported to the stratosphere (Altshuller, 1988; Hough and Derwent, 1990). Carbon dioxide concentrations have increased from about 280 μmol CO₂ mol⁻¹ to 355 μmol CO₂ mol⁻¹ over the past century and are projected to double the current levels within the next century (Post et al., 1990). Stratospheric O₃ concentrations are thought to have declined (1-3%) over the past several decades in response to increasing CFC's and N₂O concentrations; however, the increases in tropospheric O₃ (1-2% per year) are estimated to partially compensate for the loss in total O₃ (Logan, 1985; Krupa and Kickert, 1989). Tropospheric O₃ concentrations vary widely over the earth's surface and are influenced

by a number of factors including: 1) localized meteorological parameters; 2) levels of solar radiation as influenced by latitude; 3) proximity to VOC and NOx emission centers; 4) background levels of O3 precursors including VOCs and other reactive organic compounds in the air mass; and 5) long range transport processes. Readers are encouraged to consult reviews by Krupa and Kickert (1989) and Barnes and Wellburn (1998), regarding more in-depth discussions on the processes influencing CO2 and O3 levels in the atmosphere. The first part of this chapter provides information on some of the progress that has been made regarding single actions of both O3 and CO2 on growth and productivity of major crops. In the final part of this chapter, the combined actions of O3 induced stress and CO2 enrichment on growth and productivity has been discussed.

2. Ozone

Tropospheric O3 is currently among the most phytotoxic pollutants in the atmosphere. It is estimated that O3 is responsible for 90% of all crop losses due to air pollution in the USA (Heck et al., 1982; Kress et al., 1985). Over the past 30-40 years, the phytotoxic effects of O3 on plants have been extensively investigated and reviewed by many authors (Unsworth and Ormrod, 1982; Cooley and Manning, 1987; Heath, 1987; Miller, 1987, 1988; Heck et al., 1988; Krupa and Manning, 1988; Darrell, 1989; Heagle, 1989; Krupa and Kickert, 1989; Heck, 1990; Sae, 1991; Lefoh, 1992).

Many of the studies on the effects of O3 on crops, including the National Crop Loss Assessment Network (NCLAN) program (Heck et al., 1988) and the Commission of the European Communities (CEC) program (Pleijel et al., 1991) to evaluate crop losses due to air pollution in the USA and Europe, respectively, were performed in open-top chambers OTC (Heagle et al., 1973). These chambers were designed for plants to grow under different gaseous atmospheric environments in the field where O3 or any other gas is either removed from the ambient air (charcoal filtered air; CF) or added at different levels of CF or ambient air (non filtered air; NF + O3) over a period of 7 h day−1 (i.e. 09:30 AM to 04:30 PM). Some of the experiments designed to study O3 effects on crop also included other factors likely to interact with O3 such as sulfur dioxide, CO2, soil moisture and pests (Heagle et al., 1988; Mulchi et al., 1992; Manning and Tiedemann, 1995; Barnes and Wellburn, 1998).

3. Physiological Impacts of O3 Exposure

3.1. PLASMA MEMBRANE

Ozone diffuses into the leaf through the stomata (Laiik et al., 1989; Heck, 1990) where it is likely to interact with the cellular membrane and alters its normal physiological function leading to several metabolic changes (Heath et al., 1974; Porcherosiewicz and Ting, 1974; Ting et al., 1974; Miller, 1987; Runcckles and Chevone, 1992; Sanders et al., 1992). Uncertainty exists whether O3 and/or its reaction products penetrate into the cell and affect cellular organelles directly or whether the metabolic changes are due to indirect effects of O3 (Miller, 1987; Heck, 1990; Runcckles, 1992). Heath (1987) stressed that the literature is confusing regarding the type of reaction that O3 can
undergo once it reaches the humid environment inside the leaf. Several possibilities with regard to the fate of O₃ in the ambient air surrounding the leaf are described by Runeckles (1992). The relative less solubility of O₃ in contrast to CO₂ may suggest that O₃ is likely to penetrate deep into the intercellular air spaces (Taylor et al., 1988); however, the high reactivity of O₃ may limit the penetration of O₃ (Runeckles, 1992).

Lask et al. (1989) measured the O₃ uptake rates in sunflower leaves and reported that no detectable O₃ flux through the leaf was observed after O₃ enters the leaf. This led them to suggest that O₃ is rapidly decomposed in the stomatal pores or substomatal cavity causing damage to cell walls and plasmalemma.

Tingey and Taylor (1982) reviewed the literature on the potential sites for O₃ induced perturbations and concluded that membranes are the primary site of O₃ attack due to the susceptibility of the membrane bound molecules to O₃. Enzymes such as ATPase that have an active sulfhydryl group can be inactivated by O₃ due to the oxidation of the sulfhydryl group to a disulfide bridge (Heath et al., 1974; Ting et al., 1974; Heath, 1987; Miller, 1987; Runeckles and Chevone, 1992). Further, disruption of membrane function may finally lead to lysis and the release of antioxidant cell contents may act to reduce the effective dose of O₃ (Miller, 1987; Heath, 1988). Ozone can pass through the plasma membrane (Mudd, 1982); however, it is likely to react with the plasmalemma as it passes into the cell (Coulson and Heath, 1974; Heath et al., 1974).

Increased membrane permeability is likely to cause an osmotic imbalance in the cell leading to secondary O₃ damage (Heath et al., 1974; Perchorowicz and Ting, 1974; Ting et al., 1974; Sanders et al., 1992). Palisade cells of young leaves, that reached maximum expansion, were the most sensitive to O₃ damage while mature cells are more resistant to O₃ induced stress due to the deposition of suberin on the walls (Rich, 1964). Also, inherent O₃ susceptibility of different species and varieties may result from less suberin deposits on the cell walls (Tingey and Taylor, 1982).

The ability of cell membranes to recover from O₃ injury is highly dependent upon the exposure regime and it is important to make a clear distinction between chronic responses, which may be reversible (i.e. low dose over long time) and acute responses, which are likely not (i.e. high dose over short time) (Heath, 1987; Runeckles and Chevone, 1992).

Chernikova (1998) observed stimulations in leaf peroxidase (PX), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) during preflowering in soybean cv. Essex grown in OTCS in response to chronic exposures to 72.0 nmol O₃ mol⁻¹ compared to plants grown at 27.6 nmol O₃ mol⁻¹. Also, results for soybean cv. Forrest grown alongside the Essex plants failed to change activities for CAT, SOD and GR in response to the elevated O₃ treatments, thereby exhibiting lower antioxidative capacity for Forrest compared to Essex. The leaves from the Forrest plants exhibited significantly greater reductions in chlorophyll and leaf elongation than was found for Essex when grown at the elevated O₃ levels. Photosynthesis (IP) rates were likewise lower in Forrest compared to Essex under the elevated O₃ treatments when plants were grown at 355 μmol CO₂ mol⁻¹.
3.2. STOMATAL RESPONSES

Ozone uptake depends upon the concentration gradient between the ambient air and the internal leaf (Laisk et al., 1989) and the resistance to mass transfer along the diffusion path (Tingey and Taylor, 1982). Amundson et al. (1987) exposed winter wheat plants to a range of 7 h O_3 concentrations (27, 54, 76 nmol O_3 mol^-1) from anthesis until harvest and observed that flag leaf stomatal conductance (g) was significantly reduced as O_3 concentration increased. Similar results were observed by Slaughter (1987) while Rudorff et al. (1996a) only observed significant O_3 effect on reduced g, toward the end of the season. For soybeans, Mulchi et al. (1988) observed reduced g, but the results were highly variable among cultivars. Cheminova (1998) reported only slight decrease in stomatal conductance in soybean during preflowering with increased O_3 exposure; however, the magnitude in responses to elevated O_3 levels was greatly enhanced during podfill with similar results among cultivars. Decreased g, observed for plants grown under increased O_3 concentrations did not account for reduced photosynthetic rates since internal CO_2 concentration may be actually increasing due to reduced photosynthetic capacity in response to O_3 induced stress (Reich and Amundson, 1985; Miller, 1987; Lehaherr et al., 1988; Darrall, 1989; Parage et al., 1991). However, the accuracy of internal CO_2 concentration measurements for determining limitations to photosynthesis has been questioned recently by the fact that stomata do not close uniformly under drought stress, reduced irradiance (Runeckles and Chevone, 1992), or O_3 induced stress (Parage et al., 1991).

Soil water status and evaporative demand are important factors that control stomatal functioning and therefore, the rate of gas exchange between the leaf and atmosphere (Amundson et al., 1986; Kohut et al., 1987; Miller, 1987). Khout et al. (1987) exposed winter wheat plants to a range of O_3 concentration (CF air, NF+30, NF+60 and NF+90 nmol O_3 mol^-1) in two consecutive years that had different precipitation regimes (134 and 224 mm) during the months of May and June. They observed that O_3 induced reductions in grain yield were less in the dryer year and suggested that plants exposed to O_3 under reduced soil moisture have a greater degree of stomatal closure, reducing the effective dose of O_3, compared with plants exposed to well-watered conditions. Similar observations were also made by Slaughter (1987). Heagle (1989) summarized the results of 11 field studies in the NCLAN program designed to compare the effects of O_3 on well-watered plants to plants subjected to periodic drought stress. The results indicated that only under significant drought stress, were relative yield responses to O_3 affected. Plants growing under severe moisture restrictions typically show reduced responses to elevated O_3 (Mulchi, personal communication) compared to well-watered plants.

3.3. PHOTOSYNTHESIS

Photosynthesis (P) is the overall physiological parameter that is most affected by O_3 (Saxe, 1991), although the degree of impact is highly dependent upon the dose of O_3 (Runeckles and Chevone, 1992). Studies of photosynthesis are important to understand the effect of air pollutant induced stress on crop growth and production (Miller, 1988).
The sensitivity of P, to air pollutants is affected by genotype (Reich and Amundson, 1985; Miller, 1987), developmental stage (Lehnher et al., 1983) and various environmental factors such as light intensity, ambient CO2 levels, nutrient status and water availability (Darrall, 1989; Runnells, 1992). Lehnher et al. (1988) exposed wheat plants to several O3 concentrations (15, 30, 70 and 100 nmol O3 mol\(^{-1}\)) for 8 h day\(^{-1}\) and noted that P, rates were reduced mainly during anthesis, which they associated with increased stomatal conductance (g, and decreases in various components of the photosynthetic apparatus such as chlorophyll concentration, soluble protein, adenylates, RuBP regeneration and Rubisco (ribulose 1, 5-bisphosphate carboxylase/oxygenase) activity. Soybean plants exposed to chronic O3 doses also had reduced leaf P, rates with increased O3 concentrations (Reich et al., 1986; Malchi et al., 1992). Chernikova (1998) found only minimal response in P, to increased O3 exposures for soybean cultivars during preflowering; however, during podfill, P, rates declined in a linear fashion over the range of O3 levels from 27 to 60 nmol O3 mol\(^{-1}\). Reductions in grain yield in wheat in response to chronic O3 induced stress was attributed to reduced P, due to early senescence and reduced capacity of plants to provide photosynthetic assimilate to the grains (Amundson et al., 1987; Lehnher et al., 1987; Miller, 1987). Also, the decrease in photosynthetic rates paralleled the content of Rubisco (Lehnher et al., 1987) in response to premature senescence of the flag leaf triggered by O3 induced stress (Amundson et al., 1987; Lehnher et al., 1987).

Sanders et al. (1992) observed that green beans had reduced P, during chronic O3 exposure early in the growing season (0 to 44 days after emergence: DAE), but recovered over night. Later in the season (i.e. 60 DAE), photosynthetic capacity and g, gradually decreased as the severity of O3 injury increased. Electron microscope examination of O3-injured leaves revealed considerable cellular disruption including tonoplast rupture which may have caused a complete disruption of the osmotic balance within the cell inactivating the photosynthetic process.

Farage et al. (1991) exposed wheat plants to acute O3 fumigation (200 and 400 nmol O3 mol\(^{-1}\) for 4, 8 or 16 h) and observed that P, was significantly reduced at all O3 doses and concluded that the first inhibitory effect of O3 on P, is a loss of carboxylation efficiency (i.e. CO2 uptake/internal leaf CO2 concentration) due to decreased activity of Rubisco. They measured the CO2 uptake rate under CO2 saturated conditions and observed that P, was less inhibited by O3. The smaller effect of O3 on P, at saturated CO2 concentrations was related to maximum rate of RuBP regeneration. However, the authors did not make any suggestion that increased P, under CO2 saturated conditions in wheat (i.e. C, species) might be related to increased Rubisco (carboxylase) activity rather than increased RuBP regeneration. Clearly, if less RuBP is consumed in the photorespiratory process, more will be available for P, ; however, CO2 saturated conditions may not increase RuBP regeneration but will inhibit the oxygenation activity of Rubisco and consequently increase the carboxylation efficiency as noted by Farage et al. (1991). A proportional decrease in net P, rates under ambient and CO2 saturated environment in wheat exposed to chronic O3 stress was observed by Lehnher et al. (1987) which suggested that O3 is limiting net P, due to reduced Rubisco activity. Physiological and anatomical differences favour C, species to be less vulnerable than C, species to the effects of air pollutants on P, (Darrall, 1989). The highly subsidized
bundle sheath cells in C₃ species appear to protect them against the effects of phytotoxic air pollutants.

4. Growth, Biomass and Yield under O₃ Exposure

4.1. TRANLOCATION AND PARTITIONING

The effects of O₃ on biochemical and physiological processes may be reflected on growth and yield. Most O₃ plant studies have focused on crop growth and productivity in order to assess the impact of current and predicted ambient O₃ concentrations on agriculture and to establish air quality standards (Ilecki et al., 1988). Reviews on the effects of O₃ on assimilate partitioning (Cooley and Manning, 1987) and plant growth have noted that O₃ suppresses root growth proportionally more than shoot growth which is attributed to the general alteration that O₃ causes in the production and distribution of photosynthetic within the plants (Miller, 1987; Runtke and Cheeke, 1992). For example, soybean plant exposed to range of chronic O₃ concentration (20, 46, 70 and 97 nmol O₃ mol⁻¹) had increased shoot/root as O₃ concentrations increased which was attributed to lower photosynthetic production and preferential distribution of assimilates to the shoot (Endress and Grunwald, 1985). However, Amundson et al. (1986) exposed soybean plants to chronic O₃ concentrations ranging from 10 to 130 nmol O₃ mol⁻¹ and found that growth was reduced with increased O₃ concentrations but the percentage of biomass allocated to leaves, stems, or roots was not altered. Similar results, also for soybeans, were found by Leadley et al. (1990).

Cooley and Manning (1987) suggested that the relative strength of the sink determines how O₃ will affect partitioning. However, membrane malfunction caused by O₃ stress is likely to make the sink strength less significant in the partitioning process (Heath, 1988; Mulchi et al., 1992). McLaughlin and McConathy (1983) exposed bush bean plants to O₃ and observed that increased retention of assimilates in leaves was accompanied by reduced transfer of photosynthate to other parts of the plant. They suggested several possibilities by which O₃ may affect the translocation process: 1) decreased phloem loading due to either a physical or biochemical restriction; 2) increased consumption of assimilate for O₃ damage repair within the leaf itself; and 3) altered balance of source and sink due to reduced production and increased demand for assimilates by the leaves. Using ¹³C to monitor C translocation in whole plants, Pausch et al. (1996a) reported significantly positive relationships for sink strength, sink intensity and relative specific uptake for leaves treated at 25, 43 and 76 nmol O₃ mol⁻¹. Conversely, root nodule values were all inversely related to O₃ exposure, thereby illustrating reduced photosynthesis supply to roots and nodules during podfill when N demands were greatest. Plants grown under the highest O₃ exposures exhibited significantly lower N fixation by nodules (Pausch et al., 1996b). Sanders (1992) exposed green beans to chronic O₃ stress and observed cellular and chloroplast envelope rupture with large starch grains mixed within the cytoplasm. This might indicate that prior to the rupture, the cell was able to photosynthesize but unable to export the photosynthate from the chloroplast. Any decrease in transport functions in response to O₃ stress is likely to result in accumulation of photosynthate in the leaves. This, in turn, would dampen P₅ through feedback mechanisms and thereby reduce the availability of
photosynthesize to sinks such as developing pods and seeds (Mulchi et al., 1992). A decrease in C translocation from leaves in response to O, will cause a significant effect on the harvest index, which is the proportion of above ground dry matter represented by the harvestable yield, i.e. grain (Endress and Grandwold, 1985; Mulchi et al., 1995).

It is not clear whether vegetative or reproductive phase is affected more by O, (Cooley and Manning, 1987). In corn, threshold doses of O, for reductions in growth were much lower than threshold doses for reduction in yield (Heagle et al., 1979a). Ear weight of sweet corn (cv. Golden Midget) was more reduced than vegetative weight (Heagle et al., 1972). Decreased pollination under chronic O, exposure was also observed by Rudorf et al. (1996b); however, the grain yield loss was partially compensated by an increase in seed weight. Two cultivars of sweet corn exposed to ambient air in California produced different results in terms of the impact of O, on vegetative and reproductive growth (Thompson et al., 1976). The most sensitive cultivar was about equally affected by O, on the vegetative and reproductive phase while the less sensitive cultivar was primarily affected during vegetative growth (Thompson et al., 1976), suggesting that it could withstand some injury during the vegetative phase with only a small loss in yield (Heagle et al., 1979a). In wheat (Heagle et al., 1979b) and soybean (Leadley et al., 1990), losses in vegetative growth and grain yield were similar. Wheat exposed to chronic O, concentration had reduced harvest index (Fuhrer et al., 1989, 1992; Pleijel et al., 1991; Mulchi et al., 1995). Reductions in grain yield were mainly associated with decreased grain weight, indicating that decreased photosynthesize production or allocation, or accelerated senescence were the major processes involved in the O, induced stress (Kress et al., 1985; Fuhrer et al., 1989, 1992). Long-term storage and remobilization may be an important source of carbohydrates during the grain fill period when the demand for assimilate is high, but the production is low due to premature senescence of photosynthesizing leaves (McCaig and Clarke, 1982; Miller, 1988).

Slaughter et al. (1993) exposed two wheat cultivars to a range of O, treatments (CF air, NF, NF+20, NF+40 and NF+80 nmol O, mol$^{-1}$) from 10 days preanthesis until maturity and examined the effects of O, exposure on seed growth components. Increasing O, concentrations reduced seed growth rate up to 69% of the control. They suggested that reduced seed growth rate was influenced by decreased photosynthesis rates of flag leaves and heads and/or by reduced translocation rates of recently and previously accumulated photosynthesize to the seeds. Grain fill duration was not reduced in response to early senescence induced by O, therefore, grain weight reductions are likely to be attributed to reduced seed growth rate rather than grain fill duration. Unsworth et al. (1984) exposed soybean plants to a range of O, concentration (CF air, 43, 64, 77 and 104 nmol O, mol$^{-1}$) and found that seed growth rate was not affected by O, but grain fill duration was reduced by increasing O, concentrations. Number of pods and seeds per pod contributed to 40% of yield losses in the 61 and 77 nmol O, mol$^{-1}$ treatments; however, in the 104 nmol O, mol$^{-1}$ treatment, these two factors contributed to ca. 60% of the yield loss while the remaining 40% in yield loss was due to reduced seed weight.
4.2. BIOMASS AND YIELD

Most studies on the effects of \( O_3 \) on crop yield were carried out during the National Crop Loss Assessment Network (NCLAN) program which started in 1980 and ended in 1987. More than 100 journal articles were published from studies within this program whose major concern was to establish functional relationships between \( O_3 \) exposure and yield. It was shown that current ambient \( O_3 \) concentrations in the U.S. are high enough to reduce yield of major agricultural crops (Heck, 1990) and that predicted levels of \( O_3 \) are likely to have a great impact on crop production (Heagle, 1989).

The first attempt to evaluate the impact of prescribed levels of \( O_3 \) on corn production in the field was made by Heagle et al. (1972). They exposed two cultivars of sweet corn to three levels of \( O_3 \) (CF air, 50 and 100 nmol \( O_3 \) mol\(^{-1} \)) from emergence to harvest. Despite several field problems that occurred during the experiment, the authors found that the reduction in ear fill in the 100 nmol \( O_3 \) mol\(^{-1} \) treatments was the major cause for yield reduction. They suggested that differences in cultivar sensitivity might be associated with the reproductive processes necessary for successful seed set. Reduced grain yield due to reduced percentage of ear fill was also noted by Kress and Miller (1984) and Rudorff (1993). Mumford et al. (1972) exposed corn plants in the greenhouse to a range of \( O_3 \) concentrations (30, 60 and 120 nmol \( O_3 \) mol\(^{-1} \)) for 5.5 h per day during 60 days when mature pollen was harvested. They reported a significant inhibition in pollen germination, up to approximately 40% of control, for the highest \( O_3 \) level.

Bone (1982) noted in a review article that studies on the effects of \( O_3 \) on flowering and fruiting are few and that yield losses were mostly related to the effects of \( O_3 \) on photosynthesizing organs. Mulchi et al. (1988) tested 12 soybean cultivars from three different maturity groups in a two year study and observed that \( O_3 \)-related overall grain yields by 12.5%, but only three of 12 cultivars had significant grain yield reductions. Heagle et al. (1979b) exposed four wheat cultivars to chronic \( O_3 \) stress (CF air, 60, 100 and 130 nmol \( O_3 \) mol\(^{-1} \)) and observed that grain yield was reduced by 16% and 33% for the 100 and 130 nmol \( O_3 \) mol\(^{-1} \) treatments, respectively. Reductions in yield were attributed to leaf injury, reduced seed numbers and decreased weight per seed. Kress et al. (1985) exposed three winter wheat cultivars to a range of \( O_3 \) concentrations and observed that grain yield could be reduced up to 23% under 60 nmol \( O_3 \) mol\(^{-1} \) concentration. Data from this experiment showed that wheat plants are much more sensitive to \( O_3 \) than previously thought (Heagle et al., 1979b).

Kress and Miller (1983) studied the impact of a range of \( O_3 \) concentrations (CF air, 42, 64, 89 and 115 nmol \( O_3 \) mol\(^{-1} \)) on soybean and found significant linear reductions in grain yield with increased \( O_3 \) concentrations which were related to fewer filled pods per plant, fewer seeds per filled pod and smaller seeds. Similar results for soybeans were reported by Unsworth et al. (1984). Endress and Gronwald (1985) exposed soybean plants to chronic \( O_3 \) stress (CF air, 70 and 97 nmol \( O_3 \) mol\(^{-1} \)) and observed that increased \( O_3 \) concentrations reduced the availability of photosynthesize to developing pods and seeds, resulting in decreased number of pods and seed weight per plant. Similar results were reported by Mulchi et al. (1992).

Futrell et al. (1989) carried out one of the first studies in Europe to establish dose
response relationships between yield and chronic O₃ stress in spring wheat. They found similar results to those obtained earlier in the USA (Heagle et al., 1979b; Kress et al., 1985). They noted that the primary effect of O₃ on grain yield was due to reduced grain weight rather than reduced number of seeds and pointed out that episodes with high O₃ concentrations are important as they relate to crop phenology (Kress et al., 1985).

Lehnert et al. (1987) exposed spring wheat full season to a range of O₃ concentrations (CF air, 35 and 100 nmol O₃ mol⁻¹) for 8 h per day and observed that dry matter production was considerably decreased under 100 nmol O₃ mol⁻¹ concentration mainly due to reduced grain weight since straw weight was less affected by O₃. Mulchi et al. (1986) exposed six wheat cultivars to a range of O₃ concentrations (CF air, 47 and 123 nmol O₃ mol⁻¹) for 4 h per day for 5 days during anthesis and observed that the highest O₃ treatment resulted in a greater reduction in grain yield (28% of control) than above ground weight (12.6% of control). Heagle et al. (1991) exposed soybean plants to O₃ during different growth stages and noted that O₃ exposure during pod-fill stage had the greatest impact on grain yield reductions.

Wheat plants exposed to high O₃ concentrations from anthesis until harvest (Amundson et al., 1987; Slaughter, 1987; Slaughter et al., 1989, 1993) had reduced grain weight but not reduced seed number, since seed-set occurred prior to O₃ fumigation in wheat caused reduction in both seed weight and seed number (Further et al., 1989; Kress et al., 1996b).

In a two-year study, Pleijel et al. (1991) evaluated the impact of O₃ on grain yield in spring wheat and found a stable response of the plants to O₃ from year to year despite the large differences in temperature between the two years. Similar results were also noted by Fuhrer et al. (1989) in a three-year study with mean temperatures ranging from 16.2°C in 1988 to 19.6°C in 1986, suggesting that effects of O₃ on wheat may not have a significant interaction within this temperature range. Slaughter and his colleagues (1987, 1989) exposed wheat plants to chronic O₃ stress from 10 day prior to anthesis until harvest over two years which had different environmental conditions. Significant decreases in dry biomass and grain yield for the highest O₃ concentration were found during cool and wet conditions in the first year while no O₃ response occurred under the warm and dry conditions in the second year. This work suggested that wheat responses to O₃ are more strongly influenced by soil moisture than temperature.

Heggstad et al. (1988) studied the effect of water-stressed and well-watered soybean plants on grain yield and above ground dry biomass under a range of O₃ concentrations. They observed linear decrease to these plant characteristics with increased O₃ exposure. However, O₃ had a smaller impact on plants grown under low-moisture which the authors associated to a lower O₃ dose received by these plants due to stomatal closure.

Reports on the effects of O₃ on grain quality are becoming more frequent (Kress and Miller, 1983; Mulchi et al., 1986, 1988, 1995; Slaughter, 1987; Slaughter et al., 1989; Fuhrer et al., 1990, 1992; Pleijel et al., 1991; Rudorff et al., 1996c). Reductions in grain quality will add to possible negative impact of O₃ on crop production and food processing.
5. Carbon Dioxide

Carbon dioxide is the substrate for photosynthesis and is present in the atmosphere at limiting concentrations for most C₃ species. Therefore, the large increases in atmospheric CO₂ concentration that are currently being witnessed are likely to result in beneficial physiological effects on crop productivity providing that growth will not be restricted by other factors such as water availability, temperature, nutrients, phytotoxic air pollutants, etc. Many recent reviews describe responses of crops to elevated CO₂ (Kimball and Idso, 1983; Lemon, 1983; Strain and Cure, 1985; Cure and Acock, 1986; Allen, 1990; Kimball et al., 1990; Mott, 1990; Wittwer, 1990; Bowes, 1991; Lawlor and Mitchell, 1991; Woodward et al., 1991).

Increases in atmospheric CO₂ concentrations over the past century may already be influencing the output of present agricultural production; however, it is difficult to evaluate this effect. In a recent study on the effects of sub-ambient CO₂ concentrations on photosynthesis (Pₚ) rate in two cultivars of wheat, an approximately 25% increase in Pₚ was observed between the pre-industrial (283 μmol CO₂ mol⁻¹) and the present-day CO₂ concentrations (350 μmol CO₂ mol⁻¹; Polley et al., 1993).

Studies on the effects of CO₂ enrichment on plants have sometime led to inconsistent and conflicting results due to differences in CO₂ levels, exposure regime (i.e. long- and short-term studies), light level, temperature, moisture, nutritional status and growth condition (Mott, 1990; Bowes, 1991; Lawlor and Mitchell, 1991). Expected increases in crop productivity in response to CO₂ enrichment studies have been noticed under field conditions (Strain and Cure, 1985).

Forecasts of crop productivity under enriched CO₂ environment is complicated further by the climate changes predicted for different parts of the world in response to the enhancement in the "greenhouse effect" (Gates, 1983; Schneider, 1988; Idso, 1990; Lawlor and Mitchell, 1991). In order to improve the knowledge base concerning the effects of future atmospheric CO₂ concentration on society, the U.S. Department of Energy set up a Carbon Dioxide Research Program in 1978 to study the effects of long-term CO₂ enrichment on vegetation (Acock, 1990). Studies on the effects of elevated CO₂ and other environmental factors on field-grown crops are critical to assess the impact of future environmental changes on agricultural production (Krupa and Kickert, 1989; Allen, 1990; Bowes, 1991; Lawlor and Mitchell, 1991).

6. Physiological and Biochemical Effects of CO₂ Enrichment

6.1. PHOTOSYNTHESIS AND PHOTORESPiration

The effects of atmospheric CO₂ enrichment on crops are primarily dependant on the crop species (i.e. C₄ vs C₃ species). In general, increased CO₂ concentration causes increased photosynthesis and reduced stomatal conductance in crop plants. Photosynthesis in plants is the process by which light energy is converted into chemical energy and atmospheric inorganic carbon (CO₂) is converted into organic carbon with oxygen (O₂) being released to the atmosphere. Oxygen competes with CO₂ for the dual functioning enzyme ribulose bisphosphate carboxylase/oxygenase (Rubisco), in a light dependent respiratory process called photorepiration (Mulchi et al., 1971; Bowes,
1990; Taiz and Zeiger, 1990). The function of this apparent “wasteful” process in which net \( P \) is decreased in \( C \), species is still uncertain. In order to avoid photorespiration, \( C \), species developed a mechanism by which internal CO\(_2\) concentration is greatly increased, thereby greatly reducing the oxygenation activity of Rubisco at 2% O\(_2\) (Taiz and Zeiger, 1990).

Photosynthesis is stimulated in \( C \), species under increased intercellular CO\(_2\) concentrations due to increased carboxylation of Rubisco (von Caemmerer and Farquhar, 1981; Mott, 1990; Bowes, 1991; Sutt, 1991). However, sensitivity to high CO\(_2\) concentrations might be reduced over time due to saturated CO\(_2\) binding to Rubisco and limited regeneration of ribulose 1, 5-bisphosphate (RuBP) and/or inorganic phosphorus (Pi); (von Caemmerer and Farquhar, 1981; Azcon-Bieto, 1983; Pearcy and Bjorkman, 1983; Mott, 1990; Bowes, 1991; Sutt, 1991; Woodward et al., 1991). With soybean grown in OTC’s 355 vs 500 \( \mu \)mol CO\(_2\) mol\(^{-2}\), no evidence was found by Chernikova (1998) in support of reduction in \( P \) in plants grown under elevated CO\(_2\) levels. During preflowering, \( P \) rates typically increased from 20-25 \( \mu \)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\) in both cultivars under study. During podfill, \( P \) rates typically increased from 25 to 30 \( \mu \)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\) under the elevated CO\(_2\) treatment compared to ambient CO\(_2\) levels.

Rising atmospheric CO\(_2\) concentrations by 25% over the past century have likely increased \( P \) rates by decreasing photorespiration (Gates, 1983; Idso, 1990; Bowes, 1991; Lawlor and Mitchell, 1991; Polley et al., 1993). Photosynthesis rates for acclimated plants exposed to saturated CO\(_2\) concentrations may tend to return to similar \( P \) rates of plant grown at ambient CO\(_2\) concentrations (DeLucia et al., 1985); however, Sage et al. (1989) studied the \( P \) responses of five \( C \), species after acclimation to high CO\(_2\) and observed increases, no change and decreases in \( P \) rates among the species.

Havelka et al. (1984) exposed wheat plants to high CO\(_2\) treatments (1200 \( \mu \)mol CO\(_2\) mol\(^{-1}\)) in open-top chambers and observed significant increases in \( P \) rates of flag leaves at all growth stages. However, the greatest impact of CO\(_2\) enrichment on \( P \) and grain yield was noted during the seed-set period where increased \( P \) in response to CO\(_2\) enrichment caused an increase in sucrose and starch content in flag leaves in response to CO\(_2\) enrichment prior to the grain fill period and could not be linked to reductions in \( P \) rates. Yelle et al. (1989a,b) studied the effect of CO\(_2\) enrichment on two tomato species and suggested that starch and sucrose accumulation could not fully explain the decline in \( P \) rates and proposed that the main cause of the acclimation to high CO\(_2\) levels was the decline of activated Rubisco.

Azcon-Bieto (1983) studied \( P \) responses to CO\(_2\) enrichment and temperature in wheat leaves in controlled-environments and observed higher carbohydrate accumulation in leaves at 20°C than at 30°C in spite of the considerably higher \( P \) rates at 30°C and suggested that photosynthetic translocation was limited at 20°C. Respiration rates would be lower at 20°C since the Q\(_{10}\) for respiration in \( C \), species is about 2.0. Radin et al. (1987) exposed cotton plants to enriched CO\(_2\) environment (650 \( \mu \)mol CO\(_2\) mol\(^{-1}\)) in OTC and observed that \( P \) rates increased more than 70%. Acclimation was only noted at the end of the season therefore allowing plants to take full advantage of the enriched CO\(_2\) environment. The authors suggested that temperature played an important role in the acclimation process (i.e. lack of feedback inhibition) since the
considerably higher temperature observed in the field study compared to those in controlled-environments (Mauney et al., 1979) typically resulted in the highly significant increase in $P_i$ under field conditions. Rudorff et al. (1996a) reported an absence of $P_i$ response to elevated CO$_2$ in wheat on several occasions prior to anthesis which can be attributed to cooler ambient temperatures at night in combination with limited sink capacity. No such inhibitions were observed after heading. Also, limited root development due to the small pot size may also have influenced acclimation in the controlled-environment experiments (Thomas and Strain, 1991).

Regulatory feedback mechanisms buffer the initial increased $P_i$ rates under CO$_2$-saturated conditions to compensate for environmental or genetic limitation in the source/sink relationships (Azcon-Bieto, 1983; DeLucia et al., 1985; Allen, 1990; Mott, 1990; Stitt, 1991; Woodward et al., 1991). For example, a common inhibitory feedback mechanism on $P_i$ is the accumulation of starch and sucrose in the chloroplast of plants exposed to prolonged CO$_2$ enrichment (Mauney et al., 1979; Kramer, 1981; Azcon-Bieto, 1983; Havelka et al., 1984; Sage et al., 1989; Mott, 1990; Wong, 1990; Stitt, 1991). Levels of starch and sucrose were reversed upon returning plants to ambient CO$_2$ concentrations and $P_i$ rates returned to normal indicating that excessive production of photosynthesize limited $P_i$ (Sasek et al., 1985). Active photosynthesize sinks during rapid growth increase the ability of plants to draw down leaf carbohydrate supplies reducing inhibitory feedback mechanism (Havelka et al., 1984; Radin et al., 1987; Stitt, 1991; Woodward et al., 1991). Another inhibitory factor on $P_i$ rates is the regeneration of the carboxylation substrate RuBP in response to the low levels of $P_i$ in the chloroplast due to the build up of sucrose levels (Azcon-Bieto, 1983; Stitt, 1991).

In C$_4$ species (e.g. corn, sorghum and sugarcane), atmospheric CO$_2$ enrichment has little or no influence on $P_i$ rates. Roger et al. (1983) exposed corn plants in OTC to a range of suprambient CO$_2$ concentrations and measured $P_i$ rates during the early kernel development stage and did not find significant differences in response to the CO$_2$ treatment.

6.2. STOMATAL RESPONSES

There is general agreement between field and controlled-environment studies that CO$_2$ enrichment decreases stomatal conductance (g) with a consequent reduction in transpiration per leaf area unit (Lawlor and Mitchell, 1991). Water use efficiency (i.e. photosynthesize produced/unit of water transpired) is generally increased since stomatal closure induced by atmospheric CO$_2$ enrichment is likely to reduce transpiration rates more than $P_i$ rates (Kramer, 1981; Kimball and Idso, 1983; Pearcy and Bjorkman, 1983; Chaudhuri et al., 1986, 1987, 1990; King and Greer, 1986; Schonsfeld et al., 1989).

Mott (1988) studied stomatal responses to CO$_2$ (C) concentrations. The relationship between both atmospheric and intercellular CO$_2$ is approximately constant but is influenced by responses of g, and $P_i$ to C (Mott, 1990). Mott (1990) pointed out that stomatal closure in response to increased C$_4$ perhaps evolved to compensate for changes in mesophyll demand for CO$_2$ at low ambient CO$_2$ (i.e. pre-industrial CO$_2$ concentrations); therefore, it seems that if $P_i$ is still limited by CO$_2$ plants may not
regulate gas exchange efficiently under ‘artificial’ increased ambient CO₂ concentration. The mechanism for stomatal responses to C₃ is yet to be clarified (Acocq, 1990; Aitken, 1990; Mott, 1990). Soybean plants grown full-season under increasing CO₂ levels from 355 to 500 μmol CO₂ mol⁻¹ exhibited a linear decrease in gₛ with increased CO₂ concentrations with typically similar gₛ responses during both preflowering and during podfill (Chernakova, 1998).

Chaudhuri et al. (1986, 1987) exposed wheat plants to a range of CO₂ concentrations and two moisture regimes and observed that under well-watered conditions, gₛ decreased with increased CO₂ concentrations, whereas under water-stressed conditions, CO₂ levels had little effect on gₛ. However, Zakaria et al. (1993) exposed cotton plants in OTC to 350 and 500 μmol CO₂ mol⁻¹ full-season and reported significant increases in Pₛ rates at 500 μmol CO₂ mol⁻¹ at all measurements dates and reduced stomatal conductance at two of four dates (78 and 85 days after emergence). Radin et al. (1987) exposed cotton plants to enriched CO₂ treatments in OTC and found that gₛ was almost unaffected by CO₂ treatment and Pₛ rates were not limited by gₛ. Similar results were also observed in wheat plants in OTC and controlled-environment experiments by Havelka et al. (1984) and Azcon-Bieto (1983). Delucia et al. (1985) noted that in cotton plants, gₛ decreased over time but the consequent decrease in Cₛ could not fully explain the reduction in Pₛ rates.

Rogers et al. (1983) measured gₛ during the early kernel development stage of corn plants exposed to a range of suprambient CO₂ concentrations and reported significant decreases in gₛ and no change in Pₛ rates with increased CO₂ concentrations.

6.3. RESPIRATION

Conflicting reports exist concerning the effects of CO₂ enrichment on respiration (Woodward et al., 1991). Some of the increases in dry weight observed for C₃ species grown under enriched CO₂ environment have been attributed to reduced respiration (Mott, 1990). However, Azcon-Bieto (1983) observed small increases in respiratory rates in wheat flag leaves which could only account for a small portion of the considerably larger reduction in Pₛ rates under long-term exposure to CO₂ enrichment.

Ziska and Teramura (1992) measured respiration at night (2100-2400 h) and observed a lower CO₂ efflux for plants grown under high- CO₂ (660 μmol CO₂ mol⁻¹) compared to plants grown under ambient- CO₂ (360 μmol CO₂ mol⁻¹) and attributed the reductions in respiration rates to lower leaf protein contents at high CO₂ and consequent decline in protein turnover or protein production.

7. Growth, Biomass and Yield under CO₂ Enrichment

7.1. TRANSLOCATION AND PARTITIONING

The effects of CO₂ enrichment on photosynthesis (i.e., increased photoassimilate availability to sinks) and stomatal conductance (i.e., increased water use efficiency) are eventually translated into increased growth, biomass and yield. Yield increases for agricultural crops are, in many instances, proportional to general increases in biomass indicating that the additional photoassimilate produced under CO₂ enriched conditions
is proportionally distributed between reproductive and vegetative parts of the plant (Havelka et al., 1984; Cure and Acock, 1986; Mott, 1990; Lawlor and Mitchell, 1991).

Krenzer and Moss (1975) exposed wheat genotypes to 600 μmol CO₂ mol⁻¹ at different growth stages in field chambers. A major response to CO₂ enrichment was noted during the period from anthesis to physiological maturity in wheat genotypes that had more kernels/spike due to greater sink capacity to incorporate the extra photoassimilate. However, no grain yield response to CO₂ was observed for exposure during the vegetative phase (i.e. prior to seed set) which was partially attributed to the lack of CO₂ effect at low temperature (i.e. below 20°C) as was also observed by Jolliffe and Tregonning (1973).

Neales and Nicholls (1978) and Havelka et al. (1984) noted that wheat plants exposed to CO₂ enrichment prior to anthesis had significantly greater specific leaf weight (SLW) than control plants due to the higher levels of starch and sucrose. However, these levels essentially became the same during the reproductive stages due to a stronger sink. Hocking and Meyer (1991) found no consistent effect of CO₂ on SLW at anthesis for wheat plants grown under a range of nitrogen concentrations. Sage et al. (1989) noted that SLW increased in all five C₄ species in their study which was attributed to increases in starch accumulation. Similar results were found for cotton (Wong, 1990) and wheat (Schonfeld et al., 1989) suggesting that plants were unable to utilize accumulated carbohydrates in the leaves, particularly at low levels of nitrogen nutrition (Wong, 1990) and water stress (Schonfeld et al., 1989). Carbon dioxide enrichment had no significant effect on SLW for corn (Roger et al., 1983; Hocking and Meyer, 1991; Rudorff, 1993), wheat (Scout et al., 1981; Rudorff, 1993) and soybean (Rudorff et al., 1995).

7.2. BIOMASS AND YIELD

There is widespread agreement that growth, biomass and yields are increased for C₄ species grown under enriched CO₂ environment (Kramer, 1981; Kimball and Idso, 1983; Lemaux, 1983; Strain and Cure, 1985; Cure and Acock, 1986; Krupa and Kichert, 1989; Mulchi et al., 1995). Plants exposed to additional CO₂, full-season generally, exhibit enhanced growth rates (e.g. increase in leaf area index) during the early growth stages which are maintained throughout the growing season in response to higher P, rates. Also, there may be increased dry weights in response to CO₂ despite similar leaf P, rates were observed after acclimation when compared to control plants (Kramer, 1981; Cure and Acock, 1986; Mott, 1990).

Lawlor and Mitchell (1991) noted in their review that very few studies on the effects of CO₂ enrichment on crop productivity were conducted under field conditions and that considerably large differences exist between results obtained from controlled-environment and field studies (Kramer, 1981; Lawlor and Mitchell, 1991). For example, Strain and Cure (1985) reported an average of 35% increase in wheat grain yield to a CO₂ doubling concentration (i.e. 680 μmol CO₂ mol⁻¹) calculated from results of a large number of studies conducted under all type of environments, but Havelka et al. (1984) observed that grain yield was increased by only 20% in wheat plants grown in OTC at 1200 μmol CO₂ mol⁻¹. Fischer and Aguilar (1976) exposed spring wheat to
approximately 750 μmol CO₂ mol⁻¹ during different growth stages in a three-year field study and observed that grain yield was either unaffected or increased by up to 22%, depending on the year. In a controlled environment study, Hocking and Meyer (1991) reported that wheat plants produced about twice the dry matter of control plants when exposed to 1500 μmol mol⁻¹, irrespective of the nitrogen supply. Chaudhuri et al. (1990) exposed wheat plants to four levels of CO₂ (i.e. 340, 485, 660 and 825 μmol CO₂ mol⁻¹) and two moisture regimes (i.e. high- and low-water) and observed that grain yields increased 44 and 74% under high- and low-water levels, respectively, from the lowest to the highest CO₂ level; however, grain yield increases were apparently the same (∼30%) under the two moisture regimes at 485 μmol CO₂ mol⁻¹ when compared to ambient CO₂ (340 μmol CO₂ mol⁻¹).

Wheat plants exposed to enriched CO₂ environment during the period of seed set showed increased numbers of productive tillers which was likely the major reason for the significant increase in grain yield (Gifford, 1977; Sionit et al., 1981; Havelka et al., 1984; Chaudhuri et al., 1986, 1987; Hocking and Meyer, 1991) but increases in the number of spikelets per head and 1000-seed weight in wheat were minimal in response to increased CO₂ levels (Sionit et al., 1981). Mulchi et al. (1995) reported significant increase in grain yields, dry biomass, straw, harvest index and seed wt. 1000⁻¹ in wheat grown in OTC’s in response to 300 μmol CO₂ mol⁻¹ compared to ambient air (350 μmol CO₂ mol⁻¹). Carbon dioxide enrichment after anthesis produced only small increases in grain yield (Havelka et al., 1984). Somewhat different results in terms of the impact of CO₂ enrichment on growth stage and subsequent grain yields were observed by Krenzer and Moss (1975), Fischer and Aguilar (1976) and Schonfeld et al. (1989), suggesting that genotype and temperature may likewise play an important role in the effect of CO₂ enrichment on grain yield.

Small responses to CO₂ enrichment on biomass are expected for C₃ species due to their low response to CO₂ enrichment on P₅, rates (Cure and Acocock, 1986; Mott, 1990). However, Cure and Acocock (1986) listed values for increased dry matter and yield production in corn under a doubling CO₂ concentration of 9 and 29%, respectively. Corn grown in OTC under a doubling of the CO₂ concentration was reported by Roger et al. (1983) to have an increase in dry biomass of almost 50% while no significant change was observed by Rudorff et al. (1996d) and Surano and Shinn (1984) when compared to control plants. Corn plants grown from germination to 30 DAE under enriched CO₂ in controlled environments (King and Greer, 1986) and full-season (Wong, 1979) exhibited increase in dry biomass by 10 and 20%, respectively, compared to plants grown in ambient air. In a more recent study involving the interaction between CO₂ (i.e. ambient and 1500 μmol CO₂ mol⁻¹) and nitrogen (N) supply (i.e. from deficient to more than adequate) on growth and dry matter in corn, Hocking and Meyer (1991) found that corn was not sensitive to CO₂, irrespectively of the N level.

8. Combined Effects of O₃ Exposure and CO₂ Enrichment

It is widely accepted that tropospheric concentrations for both CO₂ and O₃ have increased concurrently over the past century and are expected to continue to increase at
even higher rates to level where they may have an even more significant impact on crop production than has been observed at current levels (Krupa and Kickert, 1989). As reported in the previous sections, much attention has been given to investigations concerning the effects of CO$_2$ and O$_3$ separately with respect to plant characteristics and photosynthesis. Insufficient attention has been given to the combined effects of O$_3$ and CO$_2$ on plant growth and development (Kimball, 1986; Krupa and Kickert, 1989; Allen, 1990; Ashmore and Bell, 1991; Manning and Tiedemann, 1995; Barnes and Wellburn, 1998). A growing number of studies concerned with the combined effects of long-term exposures to CO$_2$ and O$_3$ on crop growth and productivity have been recently published (Barnes and Pitman, 1992; Mulchi et al., 1992, 1995; Zakaria et al., 1994; Balague et al., 1995; Barnes et al., 1995; Ruo et al., 1995; Rudorff et al., 1995, 1996a,b,c,d; Fangmeier et al., 1996; Kramer et al., 1997; Chernikova, 1998).

Mulchi et al. (1992) were among the first to investigate the combined effects of atmospheric CO$_2$ and O$_3$ enhancement on soybean. Plants were subjected to three levels of CO$_2$ (350, 400 and 500 μmol CO$_2$ mol$^{-1}$) and three levels of O$_3$ (seasonal 7 h average of 23, 40 and 66 nmol O$_3$ mol$^{-1}$) applied singly or in combination during full-season in open-top chambers (OTC) in the field. They observed that CO$_2$ enrichment stimulated P$_r$ rates and increased soybean grain yield up to 16% while O$_3$ enhancement reduced P$_r$ rates and decreased grain yield up to 29% when compared to the control treatment (23 nmol O$_3$ mol$^{-1}$ and 350 μmol CO$_2$ mol$^{-1}$). Under both CO$_2$ and O$_3$ enrichment (500 μmol CO$_2$ mol$^{-1}$ and 66 nmol O$_3$ mol$^{-1}$), grain yield was reduced by only 13% when compared to the control indicating that the combined effect of the gases was largely additive (i.e. independent). However, at 40 nmol O$_3$ mol$^{-1}$, the high CO$_2$ level (500 μmol CO$_2$ mol$^{-1}$) totally counteracted the negative impact of O$_3$ on growth and yields. Similar results were reported by Chernikova (1998) for soybeans. In another interactive study with CO$_2$ and O$_3$, Rudorff et al. (1996a,b) exposed wheat plants during two growing seasons to two levels of CO$_2$ (350 and 500 μmol CO$_2$ mol$^{-1}$) and two levels of O$_3$ (seasonal 7 h average of 20 and 60 nmol O$_3$ mol$^{-1}$) applied singly or combination during full season in OTC. They also observed that CO$_2$ enrichment partially counteracted the damage from O$_3$ exposure since P$_r$ yield and dry biomass responses were equivalent to the control group (low O$_3$ and ambient CO$_2$). Barnes et al. (1995) exposed five wheat cultivars to two CO$_2$ concentrations (355 and 708 μmol CO$_2$ mol$^{-1}$) and two O$_3$ regimes (< 5 nmol O$_3$ mol$^{-1}$ and from 15 to 75 nmol O$_3$ mol$^{-1}$) in phytotron. They observed that reduction in growth induced by O$_3$ at elevated CO$_2$ was similar to that induced by O$_3$ under ambient CO$_2$. Therefore, it is suggested that CO$_2$ enrichment may be affording minimal additional protection against O$_3$ damage.

From these studies, it seems that CO$_2$ counteracts O$_3$ damage at moderate levels of O$_3$, exposure while acute O$_3$ exposure reduces the beneficial effect of CO$_2$ enrichment on plant growth. The role of CO$_2$ enrichment to provide substrates for detoxification and repair processes against O$_3$ damage is not clear (Polle et al., 1995; Chernikova, 1998). There is growing evidence that CO$_2$ enrichment reduces the absorbed dose of O$_3$ in response to decreased g$_s$ (Barnes and Wellburn, 1998; Chernikova, 1998); however, conflicting results are presented in the literature on this subject. In general, O$_3$ decreases g$_s$ (see previous section). Some combination studies have shown that the elevated O$_3$ environment, when enriched with CO$_2$, or vice versa, may cause further
decreases in $g$. For example, in radish, a clear additive effect of elevated $CO_2 + O_2$ on $g$ was observed after 4 weeks of exposure (Barnes and Pifermann, 1992). Also, for five wheat cultivars, a significant decrease in $g$ was observed when compared control and elevated $CO_2 + O_2$ treatments, suggesting that $CO_2$ may reduce the $O_2$ dose to plants (Barnes et al., 1995). Similar results were also observed for wheat (Rudorff, 1993; Rudorff et al., 1996a). No change in $g$ for either $CO_2$ or $O_2$ or both was noted for corn plants grown in open-top chambers (Rudorff, 1993). Studies with soybean (cv. Clark) showed that $O_2$ caused a significant decrease in $g$, but with the addition of $CO_2$, trends for continued decreases in $g$ were noted with the greatest responses appearing during preflowering (Mulchi et al., 1992). Chernikova (1998) modeled $g$ responses in soybean cv. Essex and Forrest to increases in both $CO_2$ and $O_2$. During the preflowering period, Forrest appeared to exhibit an additive response to the two gases; however, during podfill, $g$, in both cultivars declined curvilinear with respect to increased $O_2$ at all $CO_2$ levels over the range 355 to 500 mmol $CO_2$ mol$^{-1}$. Reductions in $g$ can only partially explain the reduced sensitivity on the soybean cultivars to $O_2$ under elevated $CO_2$.

In summary, $g$ for crops will be reduced under elevated $CO_2 + O_2$; however, a general statement that $CO_2$ has a protective role against $O_2$ by further decreasing $g$, and therefore, reducing the $O_2$ dose to plants is still premature. In some instances, variable results were observed for measurements performed at different growth stages as several other factors may play an important role in the stomatal behavior under elevated $CO_2$ and $O_2$. Therefore, further studies are required on this subject.

Chernikova (1998) studied the activities of antioxidative enzymes in soybean cultivars (Forrest and Essex) grown under elevated $CO_2$ and/or $O_2$. She observed reduced $O_2$ damage for cv. Forrest under elevated $CO_2$ but the activities of antioxidative enzymes remained largely unchanged. These results contradict previous results obtained by Rao et al. (1993) for spruce needles. Therefore, studies on the protective role against $O_2$ of antioxidative enzymes under CO$_2$ enriched environment are still needed. Higher levels of $CO_2$ likely increase the pools of carbohydrates for the repair of tissue damaged by $O_2$ and for substrate supply, such as ascorbate, needed to detoxify the $O_2$ products in cells.

The ability of $CO_2$ to counteract $O_2$ induced stress on plants will be determined not only by the $CO_2$ concentration and the plant response to $CO_2$ enrichment but also by the magnitude of the $O_2$ induced stress. For example, $C_3$ species are less responsive to $CO_2$ enrichment than $C_4$ species (Cure and Acock, 1986). On the other hand, Rudorff et al. (1996b) observed that in corn, a $C_4$ species, $O_2$ caused some damage to grain yield as a consequence of reduced seed set which was not counteracted by the $CO_2$ enriched environment. Fangmeier et al. (1996), in a study of the combined effects of elevated $CO_2$, $O_2$, and nitrogen on wheat in Europe, did not find a significant $O_2$ effect on yield for the cultivar Minaret, while other wheat cultivars (cv. Albis and Turbo) presented a significant impact upon comparable $O_2$ exposure (Fuhrer et al., 1992; Fangmeier et al., 1994).

The lack of interaction between $CO_2$ and $O_2$ treatments on growth and productivity suggests that both gases are acting independently. However, from a biological perspective, the independent actions of the treatment gases are difficult to accept. In general, $CO_2$ enrichment causes partial stomatal closure (Woodward et al.,...
Consequently, the uptake of phytotoxic air pollutants will be reduced under CO$_2$-induced stomatal closure, thereby causing lower air pollutant injury (Ornom, 1982; Allen, 1990; Chernikova, 1998). On the other hand, acute O$_3$ exposures can reduce the beneficial impact of CO$_2$ enrichment by decreasing photosynthesis which will further reduce internal leaf CO$_2$ concentration (Mulchi et al., 1992; Chernikova, 1998). Another aspect is that the cumulative effects of O$_3$ on photosynthesis and translocation will limit potential plant responses to CO$_2$-enrichment (Barnes and Pfrimm, 1992; Mulchi et al., 1992). Therefore, it seems that the effects of CO$_2$ and O$_3$ on vegetation, under the range of concentrations expected some time during the first half of the coming century, are likely to be counteracted by each other.

In order to better understand the impact of global change on agricultural production, it is important to concentrate research efforts in areas where uncertainties can be reduced (Schneider, 1989). This will require close co-operation and communication among scientists from multiple disciplines (Krupa and Kickert, 1989). Projections of the impact of global climate change on agriculture (Adams et al., 1990; Stockle et al., 1992) appear to be overestimated. Such projections account for the beneficial physiological effect of atmospheric CO$_2$ enrichment on plant growth based on results from plants grown under experimental conditions without considering air quality factors (Barnes and Pfrimm, 1992). Refinement of crop growth models for predictive purposes cannot be made until interactive effects of elevated CO$_2$, air quality and other important environmental factors such as water availability and temperatures are better understood (Allen, 1990; Barnes and Pfrimm, 1992; Manning and Tideman, 1995; Barnes and Wellburn, 1998).

It is considerably well established that the predicted increases in atmospheric CO$_2$ concentration are coupled with increases in tropospheric O$_3$ concentration which is expected to be 50% higher around the year 2020 (Hough and Derwent, 1990). If elevated CO$_2$ concentrations are able to partially counteract the deleterious effects of increased O$_3$ levels on crop production, we could infer that the maximum beneficial effects of predicted CO$_2$ increase on crop production may not be fully materialized, while conversely, future crop losses due to increases in tropospheric O$_3$ will not be as large as has been assessed. Such interactive effects on crop production need to be further investigated and is crucial for a better understanding of the joint effects of CO$_2$, enrichment and O$_3$ stress on crop production. It is also important to develop crop cultivars with higher total antioxidative capacities as a hedge against future losses from oxidative stress, irrespective of future changes in CO$_2$ levels.

9. Summary

Progressive changes in concentrations of tropospheric trace gases such as carbon dioxide (CO$_2$) and ozone (O$_3$) are likely to affect agricultural production. Potential deleterious effects of O$_3$ deposition on crops have been documented since the early 1950s. Ozone diffuses through the stomata into the leaf where it likely interacts with cellular membranes and alters their normal functioning. Photosynthesis is the overall physiological parameter that is most affected by O$_3$. Most studies on the effects of O$_3$ on crops were focused on crop growth and productivity. These studies showed that
current O₂ concentrations in the U.S. are high enough to reduce yields of major crops and predicted levels of O₂ are likely to have a great negative impact on agricultural production. On the other hand, it has been well documented that CO₂ enrichment has positive physiological effects on most crops, especially C₃ species, since CO₂ is often a limiting factor for photosynthesis. Even though a substantial knowledge base has been acquired concerning the single actions of both O₂ and CO₂ on crops, a limited number of studies have been devoted to the combined effects of these gases. Results from these studies have shown that enriched CO₂ environments partially counteract the deleterious effects of O₂ when applied under chronic low-level conditions. However, maximum increases in crop production in response to atmospheric CO₂ enrichment in the future are likely to be jeopardized by concurrent increases in tropospheric O₂ concentrations. The actions of each gas on the various plant processes are not expected to be independent even though their contrasting impact on growth and productivity may appear to counteract the effect of each other. Efforts to improve air quality should continue to be taken seriously and have to be effective - a difficult task - due to the constant world-wide increases in emission sources.

10. References


University of Maryland, College Park, MD.


Manning, W.J. and Tiedemann, R.J. (1995). Climate change: potential effects of increased atmospheric carbon dioxide (CO2), ozone (O3) and ultraviolet-B (UV-B) radiation on plant species. Environ. Pollut. 88, 219-245.


PLANT RESPONSES TO ELEVATED CO₂ & O₂


