http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5 (7): 798-801, 2002

Reversibility of Photosynthetic Inhibition After Long-term Exposure of Wheat Plants (*Triticum aestivum* L. Cvs. Sesquehanna and Gore) to Elevated Levels of Ozone

[1]A. A. Ali, [2]C.L. Mulchi, [3]I.A. Hassan and [1]Y.A. El-Zawahry
[1]Department of Botany, Faculty of Science, University of Zagazig, Zagazig, Sharkia, Egypt
[2]University of Maryland, Department of NRSLA, College Park, MD 20742, USA
[3]Department of Botany, Faculty of Science, University of Alexanderia, Alexanderia, Egypt

Abstract: Open-top chambers (OTC's) were conducted to determine interactive effects of atmospheric CO_2 and O_3 air pollution on photosynthetic responses in wheat. The plants were grown full-season in these chambers supplied with charcoal-filtered air (CF) as control, CF + 155µL CO_2 L^{-1} as high CO_2, non-filtered air (NF) + 40nL O_3 L^{-1} as high O_3 and NF + 155µL CO_2 L^{-1} + 40nL O_3 L^{-1} as combined high CO_2 and high O_3. Photosynthetic rates (P_n) were measured three times during vegetative and reproductive stages with portable gas exchange system (LI-COR 6200). In general, P_n rates were stimulated by high CO_2 and reduced by high O_3 but in some cases, combined high CO_2 and high O_3 increased the P_n rates. The data showed continuous increases in P_n rates during pre-flowering and early seed formation and drops during late seed formation stage. This study supports that the concentration of CO_2 at 5 ± 500µL L^{-1} treatment had a protective role against adverse impacts of O_3 exposure at concentration 5 ± 60nL L^{-1} treatments.

Key words: Photosynthesis, inhibition, wheat, cultivars, ozone

Introduction

Many models designated to assess the future impacts of complex phenomenon as climate change on crop growth and productivity have typically considered the beneficial effects of rising atmospheric CO_2 concentration on plants, while largely neglecting the potential negative impacts of phytotoxic gases such as O_3 (Adams *et al.,* 1990; Stockle *et al.,* 1992).

Carbondioxide and ozone are commonly referred to as "greenhouse" gases because of their abilities to absorb infrared radiation being emitted by earth resulting in the re-emission of this energy into the troposphere. Tropospheric O_3 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 carbon emission centers; 4) background levels of O_3 precursors including VOC's and other reactive organic compounds in the air mass; and 5) long range transport processes (Krupa and Kickert, 1989; Barnes and Wellburn, 1999). Readers are encouraged to consult reviews regarding more in-depth discussions on the processes influencing CO_2 and O_3 levels in the atmosphere.

Both CO_2 and O_3 have fundamental effects on CO_2 exchange by plants. The CO_2 uptake (photosynthesis) may be affected, with the net C gain allocated to different plant processes. In general, elevated CO_2 enhances the rate of CO_2 uptake for C_3 plants (Bowes, 1993). However, due to acclimation in some cases the rates of photosynthesis at elevated CO_2 may not be as high as expected based on ambient CO_2 plants (Amthor, 1997).

The most famous physiological parameters affected by O_3 is photosynthesis (P_n) (Saxe, 1991). Studies of photosynthesis are important to understand the effect of air pollutants including stress on crop growth and production (Miller, 1988). Lehnherr *et al.* (1988) noted that P_n rates were reduced mainly when they exposed wheat plants during senescence to several concentrations of O_3 such as 15, 30, 70 and 100 nmol O_3 mol^{-1} full season for 8 h day^{-1}. Soybean plants exposed to chronic O_3 doses also had reduced leaf P_n rates with increased O_3 concentrations (Reich *et al.*, 1986; Mulchi *et al.*, 1992). Chernikova (1998) found only minimal responses in P_n to increased O_3 exposures for soybean cultivars during pre-flowering; however, during podfill, P_n rates declined in a linear fashion over the range of O_3 levels 27 to 60 nmol O_3 mol^{-1}.

The reduction in P_n rates of bean plants during chronic O_3 exposure observed early in the growing season (0 to 44 days after emergence), but recovered over night. Later in the season, (i.e. 60 days after emergence), photosynthetic capacity and stomatal conductance gradually decreased as the severity of O_3 injury increased (Sanders *et al.*, 1992).

Photosynthesis is stimulated in C_3 species under increased intercellular CO_2 concentration due to increased carboxylation of Rubisco (Bowes, 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) (Stitt, 1991).

Few studies investigated the combined effects of O_3 and CO_2 on photosynthesis, with some suggest. McKee *et al.* (1997) reported that *Triticum aestivum* had a large increase in Rubisco activity with elevated CO_2 at high but not low O_3, and had little change in conversion activity with elevated CO_2 at either O_3 level. Similarly, Kull *et al.* (1996) indicated slightly greater increase in photosynthetic rate for *Populus tremuloides* with elevated CO_2 at low compared to high O_3, and a greater decrease in photosynthetic rate with elevated O_3 at high compared to low CO_2.

The main objective of this study was to investigate the possible interactive effects of CO_2 and O_3 on one of the biochemical responses of wheat plants grown in open-top chambers.

Materials and Methods

Design and treatments: All measurements were performed in 16 open-top chambers (OTC's) as described by Heagle *et al.* (1973) during the period of early March to late June. The studies were carried out at the South farm, United States Department of Agriculture (USDA)-Beltsville Agriculture Research Center (BARC) near Washington, DC, USA. The soil was amended with fertilizers at the rates recommended for wheat plants and pre- or post-emergence herbicide was applied to control weeds. Sprinkler irrigation units were utilized to maintain soil moisture levels near field capacity. Wheat plants were grown in plots equipped 3-m diameter x 2.5-m high open top chambers. Two replicates with 8 chamber treatments per replicate i.e. 4 air quality treatments, 2 moisture regimes and 2 cultivars. Air quality treatments are charcoal-filtered air (CF) as a control, CF + 155µL CO_2 L^{-1} (high CO_2), non-filtered air (NF) + 40nL O_3 L^{-1} (high O_3) and NF + 155µL CO_2 L^{-1} + 40nL O_3 L^{-1} (high CO_2 + high O_3). The two moisture regimes are well-watered and restricted water conditions. Wheat cultivars are gore and sesquehanna. The treatments were arranged in complete factorial design 4 x 2 x 2. The CO_2

treatments were applied from a bulk tank and injected to the OTC's box fans 18h day^{-1} (0400-2200 h EST) at rates necessary to raise the ambient CO_2 levels in the high CO_2 treatments by 155 µL CO_2 L^{-1}. The O_3 was generated from cylinder O_2 using a Griffin model FTCIA O_3 generator (Griffin Technique Co., NJ) and introduced into the blowers of the OTC between the particulate filter and fan.

Leaf photosynthetic rates (µmol m^{-2} s^{-1}): Photosynthetic rate under different treatments was measured from all chambers with a portable closed gas exchange system (Model LI-6200 primer, LI-COR, Lincolin, NE). Photosynthesis measurements where taken three times during vegetative and reproductive growth stages of wheat plants: one before flowering (pre-flowering); the second one at early seed formation; and the third one after seed formation on expanded leaves of the upper canopy under direct sun light during middle of the day. The P_n rates were always performed on three plants per cultivar per chamber between 11.00 to 14.00 h EST.

Wheat yield: It was calculated for harvested plants after reaching the maturity. By removing seeds from each plant, these seeds were left until reaching the constant weight. The collected seeds were weighed for each meter square and expressed as g/m^2.

Statistical analysis: Data was analyzed using analysis of variance (ANOVA) procedures. Treatment means were separated using least significant difference (LSD) comparisons where ANOVA's f-tests were significant (Gomez and Gomez, 1984). Significance was tested at $P \leq 0.05$ level. The software developed by the Statistical Analysis System (SAS Institute, 1990) was used to perform all analysis.

Results and Discussion

The measurements of 4-hours temperature for the air surrounding wheat plants inside the OTC's and soil temperature are shown in Fig. 1 and 2.

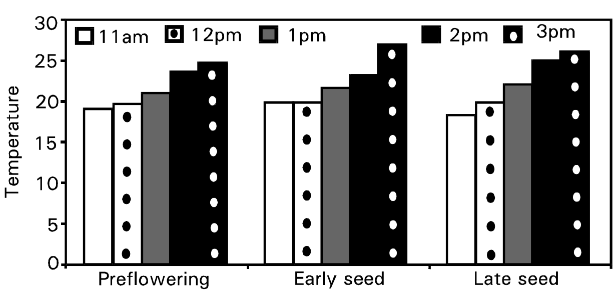

Fig. 1: Mean values of air temperature (° C) in OTC's of wheat at different growth stages under four air quality treatments and two soil moisture regimes.

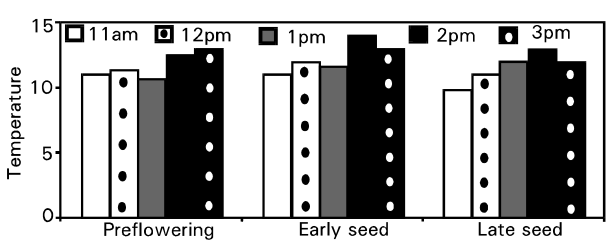

Fig. 2: Mean values of temperature (° C) in OTC's of wheat at different growth stages under four air quality treatments and two soil moisture regimes.

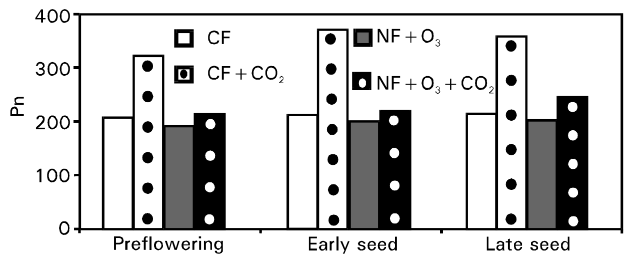

Fig. 3: Photosynthetic rates (µmole m^{-2} s^{-1}) of wheat leaves at different growth stages under four air quality treatments and wet soil.

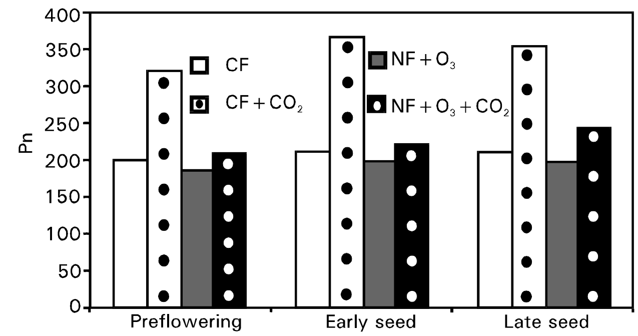

Fig. 4: Photosynthetic rates (µmol m^{-2} s^{-1}) of wheat leaves at different growth stages under four air quality treatments and dry soil.

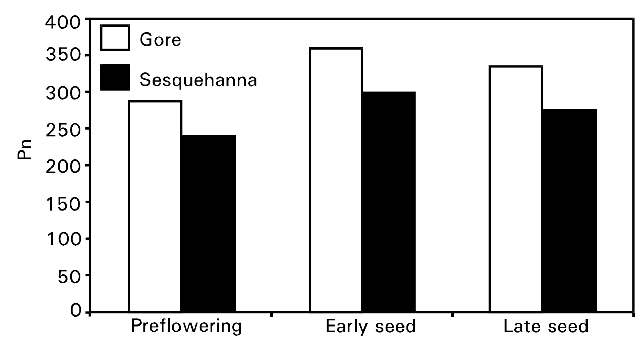

Fig. 5: Photosynthetic rates (µmol m^{-2} s^{-1}) of wheat leaves for its cultivars at different growth stages under treatments and two soil moisture regimes.

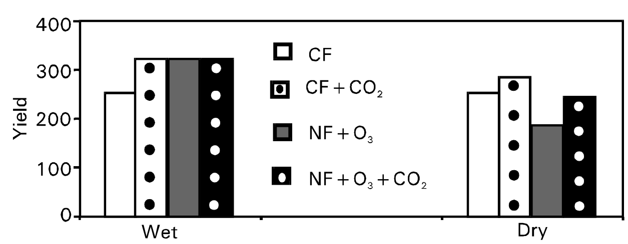

Fig. 6: Mean values wheat yield (g/m^2) under four air quality treatments and soil moisture regimes.

Gradual increase occurred in air temperature for all growth stages till reaching 2pm then slight decrease at 3pm. The soil temperature for all growth stages was not taking clear manner. Da Costa *et al.*

(1986) suggested that CO_2 release or uptake of a full crop in the field could be predicted with reasonable accuracy from knowledge of the air temperature and soil moisture content.

Effects of CO_2 and O_3 on photosynthesis P_n leaf measurements in winter at three growth stages are summarized in Fig. 3, 4 and 5, respectively. Also, these values were higher under wet conditions in comparing with that at dry treatments. The P_n rate values were increased gradually starting from pre-flowering growth stage but decreased slightly through the stage of late seed formation. Wheat cultivars exhibited big difference in P_n values for gore and sesquehanna (Fig. 5). The data showed that wheat gore was more sensitive to treatments than sesquehanna.

In general, wheat plants grown under enriched CO_2 had higher P_n rates than plants grown under ambient CO_2 (Fig. 3 and 4). In few cases, the CO_2 enrichment had no significant effect on P_n rates under dry conditions in comparison with combination between CO_2 and O_3 (Fig. 6), which may be attributed to the fact that plants were fully acclimated to CO_2 enriched environment (Allen, 1990). During the later stages of vegetative growth, perhaps CO_2 was no longer a limiting growth factor. During this period, sink capacity becomes limited and P_n rates were likely reduced due to a possible accumulation of starch grains in the chloroplast which triggers feedback mechanisms that inhibit photosynthesis (Stitt, 1991). However, during the late and early handing process significant CO_2 effects were again observed which might be attributed to the greater demand for carbohydrates in response to increased sink capacity by the plant (Woodword *et al.*, 1991; Stitt, 1991). Later in the season, when plants were in the ripening stage, no significant difference was found but plants grown under enriched CO_2 presented higher P_n rates for each of the two last readings (early seed formation and late seed formation), respectively. This can be attributed to a small delay in leaf senescence observed for plants grown under enriched CO_2. Barnes *et al.* (1995) reported that interactions between carbon assimilation, carbohydrate status and chemical composition (nutrient status) may dictate the manner in which plants respond to rising CO_2 concentrations, and governed the ability of the plant to sustain its positive response to CO_2 enrichment.

Chronic O_3 exposure tended to reduce P_n rates (Fig. 3 and 4) but the results were significant primarily when combined over dates. Significant lower P_n rates were observed at pre-flowering growth stage and at the early and late ripening stages, respectively. The overall reduction in P_n rates observed during ripening can be attributed to the early senescence noted for plant leaves exposed to chronic O_3 stress (Heck, 1990). The overall effects of O_3 on P_n rates were significant with high-O_3 treatments reducing P_n rates. Since measurements were taken preferentially on green leaves avoiding measurements on senesced leaf tissue the P_n rates on the high-O_3 treatment plants were likely over estimated.

The overall treatment effects on P_n showed that CO_2 enrichment prevented O_3 exposed plants from reduction in P_n rates below that observed from the control (Fig. 3 and 4). Photosynthetic rates for the combined enriched CO_2 level with atmospheric O_3 were significant under wet treatments for all growth stages compared with dry ones, which most probably can be attributed to the reduced temperature. The P_n rate values were significant for early and late seed formation stages in comparison with high O_3 treatments.

The sensitivity of P_n to air pollutants is affected by genotypes (Reich and Amundson, 1985; Miller, 1987), development stage (Lehnherr *et al.*, 1988), and various environmental factors such as light intensity, ambient CO_2 levels, nutrient status and water availability (Darral, 1989; Runeckles, 1992). The reduction in P_n rates of wheat plants during senescence are associated with increased stomatal conductance and decreased 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. Farage *et al.* (1991) concluded that the first inhibitory effect of O_3 on P_n is the loss of carboxylation efficiency (i.e. CO_2 uptake/ internal leaf CO_2 concentration) due to decreased activity of Rubisco.

The effects of CO_2 and O_3 on wheat yield quality are illustrated in Fig. 6. Both gases were found to cause large changes in grains quality of wheat. The elevated CO_2 significantly increased the wheat yield while high O_3 reduced the quality. The interactive effect of high CO_2 and high O_3 exhibited significant increase in grain quality of wheat in comparison with atmospheric O_3 treatments. Reduction in grain yield of wheat in response to O_3 levels induced stress was attributed to reduced P_n due to early senescence and reduced capacity of plants to provide photosynthetic assimilate to grains (Amundson *et al.*, 1987; Lehnherr *et al.*, 1987; Miller, 1987). Also, the decrease in photosynthetic rates paralleled the content of Rubisco (Lehnherr *et al.*, 1987) in response to premature senescence of the flag leaf triggered by O_3-induced stress (Amundson *et al.*, 1987; Lehnherr *et al.*, 1987). Kull *et al.* (1996) explained the impact of O_3 on plants by depression of photosynthetic activity and the accelerated senescence of leaves. Moreover, a genotype considered tolerant with normal CO_2 levels appears to have decreased O_3 tolerance with elevated CO_2.

Electron microscope examination of O_3 injury revealed that the considerable disruption including tonoplast rupture which may have caused a complete disruption of the osmotic balance within the cell inactivating the photosynthetic process (Sanders *et al.*, 1992).

There was no evidence of an interactive effect as elevated CO_2 increased and high O_3 decreased the photosynthesis to a similar extent at both levels of the other gas on *Triticum aestivum* (Rudorff *et al.*, 1996). Thus, the response to O_3 and CO_2 likely was additive in these species, with the response to O_3 and CO_2 at least partially canceling each other out. *Picea abies* showed little effect of elevated CO_2 on photosynthesis at either O_3 levels or an increase in photosynthesis at both CO_2 levels (Lippert *et al.*, 1997). The wheat plant (C_3) species exhibited significant response to the atmospheric CO_2 and O_3 treatments. The mechanism(s) involved concerning a possible protective role of CO_2 against O_3 are largely unknown. In addition, the results from present study support a hypothesis that the added CO_2 somehow protects the wheat's ability to partition the photosynthates to developing sinks as grains. The collected data supported that the elevated CO_2 treatments can increase the productivity of wheat plant.

References

Adams, R.M., C. Rosenzweig, R.M. Peart, J.T. Ritchie, B.A. McCarl, J.D. Glyer, R.B. Curr, J.W. Jones, K.J. Boote and L.H. Allen, 1990. Global climate change and US agriculture. Nature, 345, 219-224.

Allen, L.H., 1990. Plant responses to rising carbon dioxide and potential interactions with air pollutants. J. Environ., Qual., 19: 15-34.

Amthor, J.S., 1997. Plant Respiratory Responses to Elevated Carbon Dioxide Partial Pressure. In: Allen *et al.* (eds.) Advances in Carbon Dioxide Effects Research. ASA Special Publication Number 61, Madison, WI., 35-77.

Amundson, R.G., R.J. Kohut, A.W. Schoettle, R.M. Raba and P.B. Reih, 1987. Correlative reductions in whole-plant photosynthesis and yield of winter wheat caused by ozone. Physiol. Biochem., 77: 75-79.

Barnes, J.D. and A. Wellburn, 1999. Air pollutant combinations. In: L. J. De Kok and Stulen I. (eds.) Responses of plant metabolism to air pollution and global change. SBP Academic Publication. The Leiden, The Netherlands.

Barnes, J.D., T. Pfirrmann, K. Steiner, C. Lutz, U. Busch, H. Kuchenhoff and H.D. Payer 1995. Effects of elevated CO_2, Elevated O_3 and potassium deficiency on Norway spruce [*Picea abies* (L.) Karst]: seasonal changes in photosynthesis and non-structural carbohydrate content. Plant, Cell and Env., 18: 1345-1357.

Bowes, G., 1991. Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. Plant, Cell and Env., 14: 795-806.

Bowes, G., 1993. Facingthe inevitable: plants and increasing atmosphere CO_2. Ann. Rev. Plant Physiol. Pl. Mol. Biol., 44: 309-332.

Chernikova, T., 1998. Ozone effect on growth, physiological characteristics and antioxidant enzymes in soybean cultivars exposed to ambient and elevated carbon dioxide. Ph.D. Dissertation. University of Maryland, College Park, MD.

Da Costa, J.M.N., N.J. Roseberg and S.B. Verma, 1986. Joint influence of air temperature and soil moisture on CO_2 release by a soybean crop. Agric. Forest Meteorol., 37: 219-227.

Darral, N.M., 1989. The effect of air pollutants on physiological processes in plants. Review. Plant, Cell and Environ., 12: 1-30.

Farage, P.K., S.P. Long, E.G. Lechner and N.R. Baker, 1991. The sequence of change within the photosynthetic apparatus of wheat following short-term exposure of ozone. Pl. Physiol., 95: 529-535.

Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for agriculture research. John Wiley, NY.

Heagle, A.S., D.E. Body and W.W. Hech, 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Env. Qual., 20: 562-570.

Heck, W.W., 1990. Assessment of crop losses from air pollutants in the United States. In: MacKenzie J.J., El-Ashry M. T. (eds.) Air pollution's toll on forests and crops, pp: 235-315. Yale Univ. Press, London.

Krupa, S.V. and R.N. Kickert, 1989. The greenhouse effect: impact of ultraviolet-B (UV-B) radiation, carbon dioxide (CO_2) ozone (O_3) on vegetation. Env. Pollut., 61: 263-396.

Kull, O., A. Sober, M.D. Coleman, R.E. Dickson, J.G. Isebrands, Z. Gagnon and D.G. Karnosky, 1996. Photosynthetic responses of aspen clones to simultaneous exposures of ozone and CO_2. Can. J. For. Res., 26: 639-648.

Lehnherr, B., F. Machler, A. Grandjean and J. Fuhrer, 1988. The regulation of photosynthesis in leaves of field-grown spring wheat (*Triticum aestivum* L., cv Albis) at different levels of ozone in ambient air. Plant Physiol., 88: 1115-1119.

Lehnherr, B., A. Grandjean, F. Machler and J. Fuhrer, 1987. The effects of ozone in ambient air on ribulose bisphosphate carboxylase/ oxygenase activity decreases photosynthesis and grain yield in wheat. J. Plant Physiol., 130: 189-200.

Lippert, M., K. Steiner, T. Pfirrmann and H.D. Payer, 1997. Assessing the impact of elevated O_3 and CO_2 on gas exchange characteristics of differently K supplied clonal Norway spruce trees during exposure and the following season. Trees Struct. Funct., 11: 306-315.

McKee, I.F., J.F. Bullimore and S.P. Long 1997. Will Elevated CO_2 Concentrations Protect the Yield of Wheat from O_3 Damage? Plant Cell Env., 20: 77-84.

Miller, J. E., 1987. Effects of ozone and sulfur dioxide stress on growth and carbon allocation in plants. In: Saunders J. A., Kosak-Channing L., Conn E. E. (eds.), Recent advances in physiochemistry. Plenum Press, New York, 21: 55-100.

Miller, J.E., 1988. Effects on photosynthesis, carbon allocation, and plant growth. In: Heck W. W., Taylor O. C., Tingey D. T. (eds.) Assessment of crop loss from air pollutants. Proceedings of International Conference, Raleigh, NC, USA. Elseiver Applied Science, London, 285-314.

Mulchi, C.L., L. Slaugter, M. Saleem, E.H. Lee, R. Pausch and R. Rowland, 1992. Growth and physiological and physiological characteristics of soybean in open-top chambers in response to ozone and increased atmospheric CO_2. Agric. Ecosys. Env., 38: 107-118.

Reich, P.B. and R.G. Amundson 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science, 230: 566-570.

Reich, P.B., A.W. Schoettle, R.M. Raba and R.G. Amundson, 1986. Response of soybean to low concentrations of ozone: I. Reductions in leaf and whole plant net photosynthesis and leaf chlorophyll content. J. Env. Qual., 15: 31-36.

Rudorff, B.F.T., C.L. Mulchi, E.H. Lee, R.A. Rowland and R. Pausch, 1996. Photosynthetic characteristic in wheat (*Triticum aestivum*) exposed to elevated O_3 and CO_2. Crop Sci., 36: 1247-1251.

Runeckles, V.C., 1992. Uptake of ozone by vegetation. In: Lefhon A. S. (ed.), Surface level ozone exposures and their effects on vegetation. Lewis Publishers, Chelsea, MI., 157-270.

Sanders, G.E., J.J. Colls and G. Clark, 1992. Physiological changes in *Phaseolus vulgaris* in response to long-term ozone exposure. Ann. Bot., 69: 123-133.

SAS Institute 1990. PC-SAS User's guide. Version 6.04 ed. SAS Institute, Cary, NC.

Saxe, H., 1991. Photosynthesis and stomatal responses to polluted air, and the use of physiological and biochemical responses for early detection and diagnostic tools. In: Callow J. A. (ed.) Advances in botanical research. Academic press, San Diego, CA. 18: 2-127.

Stitt, M., 1991. Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant, Cell and Env., 14: 741-762.

Stockle, C.O., P.T. Dyke, J.R. Williams, C.A. Jones and N.J. Rosenberg, 1992. A method for estimating the direct and climate effects of rising atmosphere carbon dioxide on growth and yield of crops. II. Sensitivity analysis at three sites in the Midwestern USA. Agril. Sys., 38: 239-256.

Woodword, F.I., G.B. Thompson and I.F. Mckee, 1991. The effects of elevated concentration of CO_2 on individual plants, populations, communities and ecosystems. Ann. Bot., 67: S-1, 23-38.