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Interactive Effects of High CO\textsubscript{2} and SO\textsubscript{2} on Growth and Antioxidant Levels in Wheat

By

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With 3 Figures

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Key words: Triticum aestivum, ascorbic acid, chlorophyll fluorescence, glutathione, high CO\textsubscript{2}, photosynthesis, SO\textsubscript{2}.

Summary

RAO M. V. & DE KOK L. J. 1994. Interactive effects of high CO\textsubscript{2} and SO\textsubscript{2} on growth and antioxidant levels in wheat. - Phyton (Horn, Austria) 34 (2): 279–290, 3 figures. - English with German summary.

The impact of elevated CO\textsubscript{2} and/or SO\textsubscript{2} on the growth and antioxidant levels of wheat (Triticum aestivum L. cv. Urban) plants has been studied. High CO\textsubscript{2} (0.7 ml l\textsuperscript{-1}) significantly enhanced shoot biomass and photosynthetic capacity, while exposure to SO\textsubscript{2} (0.14 µl l\textsuperscript{-1}) resulted in a decreased shoot biomass and in an injured photosynthetic apparatus, illustrated by a loss of chlorophyll and a decreased ratio of variable to maximal fluorescence (F\textsubscript{v}/F\textsubscript{m}) and A\textsubscript{max}. However, combined exposure of plants to high CO\textsubscript{2} and SO\textsubscript{2} eliminated the negative effects of SO\textsubscript{2}. Sulfate accumulation was almost equal in plants exposed to SO\textsubscript{2} and, high CO\textsubscript{2} and SO\textsubscript{2}. A significant increase in ascorbate, glutathione and their redox state was observed in plants exposed to high CO\textsubscript{2} and SO\textsubscript{2}, compared to that of plants exposed to solely SO\textsubscript{2}. The absence of the negative effects of SO\textsubscript{2} in the presence of high CO\textsubscript{2} might be related to a high redox state of ascorbate and glutathione.

Abbreviations: A\textsubscript{max}, maximum rate of oxygen evolution at saturated light and CO\textsubscript{2} (µmol m\textsuperscript{-2} s\textsuperscript{-1}); ASA, reduced ascorbic acid; DHA, dehydroascorbic acid; F\textsubscript{m}, maximum emission of photosystem-II chlorophyll fluorescence; F\textsubscript{v}, variable component of F\textsubscript{m}; GSH, reduced glutathione; GSSG, oxidized glutathione.

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Zusammenfassung


Es wurde der Einfluß von erhöhtem CO$_2$ und/oder SO$_2$ auf das Wachstum und den Antioxidantiengehalt von Weizenpflanzen (Triticum aestivum L. cv. Urban) untersucht. Hohes CO$_2$ (0,7 ml l$^{-1}$) erhöht signifikant die Sproßbiomasse und die Photosynthesekapazität, während eine Begasung mit SO$_2$ (0,14 μL l$^{-1}$) zu einer Veränderung in der Biomasse und zu einem beeinträchtigten Photosyntheseapparat führt, was durch einen Verlust an Chlorophyll und ein vermindertes Verhältnis von variabler zu maximaler Fluoreszenz ($F_v/F_m$) und $A_{max}$ verdeutlicht wird. Eine Begasung von Pflanzen mit hohen CO$_2$- und SO$_2$-Mengen jedoch verhindert die negativen Einflüsse von SO$_2$. Die Sulfatanreicherung in den Pflanzen war ziemlich gleich bei SO$_2$ und hohem CO$_2$ und SO$_2$-Einfluß. Ein signifikanter Anstieg an Ascorbat, Glutathion und deren Redoxzustand wurde in Pflanzen beobachtet, welche mit hohem CO$_2$ und SO$_2$ behandelt wurden gegenüber solchen, welche bloß SO$_2$ ausgesetzt waren. Das Fehlen von negativen Effekten durch SO$_2$ in Anwesenheit von hohem CO$_2$ dürfte auf einen hohen Redoxzustand von Ascorbat und Glutathion zurückzuführen sein.

Introduction

Due to the increasing energy needs of an expanding human population, atmospheric levels of carbon dioxide (CO$_2$) are expected to double during 21st century (LONG & al. 1993). This, in turn, has prompted the evaluation of many plant species for their response to elevated CO$_2$ concentrations (EAMUS & JARVIS 1989, SAGE & al. 1989, YELLE & al. 1989, ZISKA & al. 1991, LONG & DRAKE 1991, BOWES 1993). Although there is a wide variation in the response of plant species, generally high CO$_2$ levels result in an enhanced plant yield. Stimulation of growth by high CO$_2$ is usually temporary and strongly dependent on the level of mineral nutrition (STULEN & DEN HERTOG 1993, STULEN & al. 1993). In industrialized and populated areas, high CO$_2$ levels are accompanied with gaseous pollutants like NO$_x$, SO$_2$ and O$_3$ (CARLSON & BAZZAZ 1985, ROZEMA 1993). This has prompted wide spread concern to evaluate the impact of high CO$_2$ and air pollutants (CARLSON 1983, BARNES & PFIRRMANN 1992, MULCHI & al. 1992).

Exposure of plants to high CO$_2$ usually enhances photosynthesis while transpiration is often decreased, due to a reduced stomatal opening (ALLEN 1990). It is generally considered that the partial stomatal closure in response to elevated CO$_2$ will reduce the impact of toxic air pollutants like SO$_2$ and O$_3$ (ALLEN 1990). However, the present experimental data on the combined effects of CO$_2$ and air pollutants are too inconsistent for the support of this assumption. Recent studies on the impact of high CO$_2$ and air pollutants have suggested that the elimination of air pollutant toxicity at high CO$_2$ levels to be due largely to the internal detoxification mechanisms rather than to a reduced pollutant uptake (KROPFF 1987, MULCHI & al. 1992, BARNES & PFIRRMANN 1992).
The physiological processes underlying the phytotoxicity of SO$_2$ are rather unclear. Sulfite ions formed due to the hydration of SO$_2$ in cell sap are very reactive and the reaction with various cellular compounds is believed to be the major cause of SO$_2$ phytotoxicity (DE KOK 1990). On the other hand, it has been proposed that the toxicity of SO$_2$ is due to the formation of superoxide anions and hydrogen peroxide (H$_2$O$_2$) generated during the oxidation of sulfite to sulfate (MADAMANCHI & ALSCHER 1991, RAO 1992). In this view, plants tolerate SO$_2$ by scavenging the toxic oxygen free radicals through a sequence of events involving metabolites like ascorbate and glutathione and the enzymes like glutathione reductase, ascorbate peroxidase and dehydroascorbate reductase (RAO & DUBEY 1993).

It has been suggested that the higher availability of carbohydrates in plants exposed to high CO$_2$ would detoxify air pollutants (CARLSON & BAZZAZ 1985, LONG & al. 1993). However, this assumption lacks experimental evidence. Synthesis of ascorbic acid, a major antioxidant, depends on carbohydrate availability (LOEWUS & al. 1990). Since plants growing under elevated CO$_2$ have high production of carbohydrates (YELLE & al. 1989), we hypothesized that growth under elevated CO$_2$ and SO$_2$ would enhance ascorbic acid to detoxify SO$_2$. A series of experiments were conducted to investigate the effects of high CO$_2$ and/or SO$_2$ on biomass production, A$_{max}$, ascorbic acid, glutathione and their redox state in an attempt to test the hypothesis and to offer physiological explanations for the observed protection in wheat plants, if any.

**Materials and Methods**

Winter wheat (*Triticum aestivum* L. cv. Urban) was germinated in plastic pots (1 l; five seeds per pot) containing commercial soil (Florafleur Potting Soil, Neveama, Zwolle, The Netherlands). Day and night temperatures were 19 and 15 °C, respectively, RH was 60–70 % and the photoperiod was 14 h at a fluence rate of 200 µmol m$^{-2}$ s$^{-1}$ (400–700 nm). Seven day old seedlings were thinned to three plants per pot and were transferred to the experimental cabinets.

Plants were exposed to high CO$_2$ and/or SO$_2$ in 150 l cylindrical stainless steel cabinets with polycarbonate tops (for a description see MAAS & al. 1987). Pressurized CO$_2$ and/or SO$_2$ diluted with nitrogen (1 ml l$^{-1}$) were mixed with air to obtain desired concentrations (715 µl l$^{-1}$ CO$_2$ and or 0.138 µl l$^{-1}$ SO$_2$) by ASM electronic mass flow controllers (Bilthoven, The Netherlands) and injected into the cabinets through a teflon pipe system. The air exchange in the cabinets was 50 l min$^{-1}$ and the air inside the cabinets was continuously circulated by a ventilator (air movement capacity 20 l s$^{-1}$) to reduce the boundary layer surrounding the leaves. The air temperature was 24 ± 2 / 18 ± 2 °C (day/night) and the RH was 68 ± 4 / 55 ± 6 % (day/night). The photoperiod was 14 h and the light intensity at plant height was in the range of 275–300 µmol m$^{-2}$ s$^{-1}$ (within the 400–700 nm range). A Philips HPI-T 400 W lamp was used as a light source.

CO$_2$ concentrations in the air stream were measured periodically with an IRGA (ADC Model 225, MK 3, Hoddesdon, UK) and the SO$_2$ concentrations in the cabinets
were estimated according to MAAS & al. (1987). The mean day/night CO$_2$ concentrations in the cabinets were 365/374 (ambient) and 715/726 μl l$^{-1}$ (high). The mean SO$_2$ concentrations were 0.004 (ambient) and 0.138 μl l$^{-1}$ (high) which remained constant during day and night. Plants were irrigated daily with tap water and on alternate days with half strength Hoagland nutrient solution (for composition see SMAKMAN & HOFSTRA 1982). To minimize spatial effects, within a cabinet, plants were randomly relocated on alternate days. A total of four cabinets were employed in the present investigation and the experiment was repeated in another set of four cabinets.

To determine whether the growth of wheat plants under high CO$_2$ for four weeks resulted in photosynthetic desensitization, the maximal photosynthetic capacity ($A_{max}$) was measured with an oxygen electrode (Model LD-2, Hansatech, Kings Lynn, Norfolk, UK). The details of the method are described elsewhere (DIJKSTRA & LAMBERS 1989). Measurements were made at 25°C at a saturating light intensity of 1600 μmol m$^{-2}$ s$^{-1}$ in the presence of 5% CO$_2$ (from a 1 M Na$_2$CO$_3$/NaHCO$_3$ buffer, 1:19 v/v). PPFD values greater than 1600 μmol m$^{-2}$ s$^{-1}$ did not increase the rates of O$_2$ evolution; consequently 1600 μmol m$^{-2}$ s$^{-1}$ was considered as saturating. O$_2$ evolution measurements were made on 4 leaf discs sampled from recently developed leaf (70-80% of total leaf area). Chlorophyll was determined in the leaf discs used in O$_2$ evolution measurements according to LICHTENTHALER & WELLBURN 1983. Whole shoot dry weight was determined after drying for 36 h at 70°C.

Chlorophyll fluorescence ratio ($F_v/F_m$) was determined from the upper leaf surfaces of 15 min dark adapted leaves using a pulse amplitude modulation fluorescence meter (PAM-101, H. Walz, Effel trich, FRG). The PPFD of modulation light was about 0.2 μmol m$^{-2}$ s$^{-1}$. After measuring $F_o$, $F_m$ was measured by exciting the surface with white light of 300 μmol m$^{-2}$ s$^{-1}$. No further increases in the ratio of $F_v/F_m$ were noted when the length of dark adaptation was increased beyond 15 min or if the actinic excitation beam increased above 300 μmol m$^{-2}$ s$^{-1}$. $F_v/F_m$ measurements were made on 4–6 leaves sampled from plants exposed to different CO$_2$ and/or SO$_2$ concentrations.

Sulfate was estimated refractometrically after separating the anions by HPLC as described by MAAS & al. 1986. The HPLC system consisted of a Kratos spectroflow pump, model 400 (Ramsey, NJ 07446, USA), provided with a Rheodyne sample injector, model 7175 (loop volume 20 μl; Cotati, CA 94928, USA) and a Knauer differential refractometer, model 9800 (Bad Homburg, Germany). The anions are separated on an Ionospher$^\text{tm}$-A anion exchange column (250×4.6 mm) with guard column (75×2.1 mm) (Chrompack, Middelburg, The Netherlands). Potassium biphthalate (30 mM, pH 4.0) was used as a mobile phase. The flow rate was 1 ml min$^{-1}$ and the column and detector are thermostated at 24°C by a water bath. The HPLC was connected with a Shimadzu Chromtopac C-RIB data processor (Kyoto, Japan). Analyses of sulfate and nitrate were made by extracting three individual whole shoots (1 g 4 ml$^{-1}$ extraction medium) from each treatment and each extract yielded five observations.

Ascorbate (ASA) and dehydroascorbate (DHA) and glutathione (GSH and GSSG) were determined as described by LAW & al. 1983 and SMITH 1985, respectively. Foliar samples (0.2 g) frozen in liquid nitrogen were extracted in 2 ml of 10 % TCA (w/v). The pH of the extract was brought to 5.6 with 10 % sodium citrate (w/v) before analyses were made. All measurements were made on recently developed leaves (70–80% of total leaf area) of six plants from each treatment.
Results and Discussion

Substantial changes were evident in the shoot biomass of plants transferred into the cabinets enriched with CO$_2$ and/or SO$_2$ when compared to that of plants exposed to ambient CO$_2$ (Fig. 1). Exposure of plants to high CO$_2$ or SO$_2$ for one week had no significant effect on whole shoot fresh weight. Exposure to high CO$_2$ for four weeks enhanced fresh weight by 24%, while exposure to SO$_2$ resulted in a decreased shoot fresh weight by 42% (Fig. 1). However, when plants were exposed to high CO$_2$ and SO$_2$ in combination, the toxic effects of SO$_2$ were eliminated and the shoot fresh weight was almost equal to that of plants exposed to high CO$_2$ (23% higher than that of plants exposed to ambient CO$_2$) (Fig. 1). Similarly, data on the dry matter production presented in Fig. 1b also indicated the deleterious effect of SO$_2$ as well the absence of SO$_2$ effect on the dry matter production of wheat plants exposed to high CO$_2$ and SO$_2$ in combination.

The observed increases and decreases were consistent with previous results obtained at high CO$_2$ (YELLE & al. 1989, HOCKING & MEYER 1991) and at high SO$_2$ (SOLDATINI & al. 1992, RAO & DUBEY 1993). Also, the observation that exposure of plants to a combination of high CO$_2$ and SO$_2$ may eliminate negative effects of SO$_2$ is consistent with the results reported earlier by CARLSON & BAZZAZ 1982 and SANDHU & al. 1992.

The negative effects of SO$_2$ on biomass production may partly be explained by negative and injurious effects of SO$_2$ on the photosynthetic apparatus. The chlorophyll content of SO$_2$ exposed plants decreased by
42% compared to plants exposed to ambient CO$_2$. However, the chlorophyll content of plants exposed to combination of high CO$_2$ and SO$_2$ was almost equal to that of plants exposed to ambient or high CO$_2$ (Tab. 1).

Similarly, the chlorophyll fluorescence ratio, $F_v/F_m$, a measure of photochemical efficiency, was significantly lower in plants exposed to high SO$_2$, while it remained unaffected in plants exposed to high CO$_2$ or to a combination of CO$_2$ and SO$_2$ (Tab. 1). Apparently, the decreases in $F_v/F_m$ ratio in SO$_2$ exposed plants appears to be directly related to the decreases in chlorophyll content (ADAMS & al. 1989, SCHMIDT & al. 1990). The inju-

Table 1

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>AA</th>
<th>EA</th>
<th>AE</th>
<th>EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll (mg g$^{-1}$ f.wt.)</td>
<td>1.83 ± 0.12</td>
<td>1.86 ± 0.15</td>
<td>1.06 ± 0.15</td>
<td>1.79 ± 0.14</td>
</tr>
<tr>
<td>$F_v/F_m$</td>
<td>0.82 ± 0.06</td>
<td>0.85 ± 0.07</td>
<td>0.70 ± 0.09</td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td>$A_{max}$ (µmol O$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>29.5 ± 2.6</td>
<td>35.4 ± 2.8</td>
<td>16.5 ± 1.6</td>
<td>33.9 ± 3.1</td>
</tr>
<tr>
<td>Sulfate (µmol g$^{-1}$ f.wt.)</td>
<td>3.8 ± 0.4</td>
<td>3.7 ± 0.4</td>
<td>13.3 ± 3.1</td>
<td>11.3 ± 2.3</td>
</tr>
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</table>

AA, 365 µl l$^{-1}$ CO$_2$ + 0.004 µl l$^{-1}$ SO$_2$
EA, 715 µl l$^{-1}$ CO$_2$ + 0.004 µl l$^{-1}$ SO$_2$
AE, 365 µl l$^{-1}$ CO$_2$ + 0.138 µl l$^{-1}$ SO$_2$
EE, 715 µl l$^{-1}$ CO$_2$ + 0.138 µl l$^{-1}$ SO$_2$

rious effects of SO$_2$ exposure were also evident from the measurements on maximum photosynthetic capacity ($A_{max}$). The $A_{max}$ of plants exposed to either high CO$_2$ or to a combination of high CO$_2$ and SO$_2$ was significantly higher (20%) compared to plants exposed to ambient CO$_2$, while in SO$_2$ exposed plants, $A_{max}$ was decreased by 40% (Tab. 1).

Generally, high CO$_2$-induced stomatal closure has been largely believed to reduce pollutant flux and protect plants from pollutant injury (ALLEN 1990). In general, SO$_2$ exposure results in increased sulfate levels of the leaf tissue and is largely dependent on the SO$_2$ concentration and duration of the exposure (DE KOK 1990). Sulfate levels in the plant are a reflection of various dynamic parameters, e.g. sulfate uptake by the root, its translocation to the shoot, and the rate of its assimilation (DE KOK
Therefore, increases in sulfate levels upon SO2 exposure can not directly be used as a measure for SO2 uptake by the plant, yet they have an indicative value. Exposure of wheat plants to high SO2 or to a combination of high CO2 and SO2 resulted in a three-fold increase in sulfate levels compared to that of plants exposed to ambient or high CO2 (Tab. 1). From these results, it is clear that the absence of toxic effects of SO2 at high CO2 can not solely be attributed to the decreased pollutant flux and that the protection is dependent on the internal detoxification mechanisms.

It is assumed, generally, that the ascorbate-glutathione cycle plays an active role in protecting plant chloroplasts from oxygen free radicals. Enhanced sulfite levels in SO2 exposed plants are believed to strongly induce oxygen free radicals. However, the significance of sulfite-induced formation of oxygen species in situ at realistic SO2 levels remain unclear as well the role of ascorbate-glutathione cycle (DE KOK & STULEN 1993). Yet, it has been proposed that high levels of ascorbate and glutathione would have adaptive value in protecting plants from the harmful effects of SO2 enhanced oxygen free radicals (RAO & DUBEY 1993). In addition, the ability of plants to maintain high ASA/DHA and GSH/GSSG ratios have been reported to determine the plant response to various oxidative stresses (CREISSON & al. 1994).

Total ascorbic acid level (ASA + DHA) was slightly higher in plants exposed to high CO2 compared to plants exposed to ambient CO2 (Fig. 2A). Although the ASA + DHA level tended to be slightly higher in plants exposed to high CO2 than in plants exposed to ambient CO2, there were no significant changes in the ASA/DHA ratio (Fig. 2B). Although there was an initial increase in the ASA + DHA level of plants exposed to SO2 for one week (P < 0.05), four week exposure decreased the ASA + DHA.
level by 10% compared to that of plants exposed to ambient CO₂ (Fig. 2A). However, exposure to high CO₂ and SO₂ in combination enhanced the ASA + DHA level by 23% compared to that of plants exposed to ambient CO₂ (Fig. 2A). In addition to changes in the ASA + DHA level, significant changes were apparent in the ASA/DHA ratio in plants exposed to CO₂ and or SO₂ (Fig. 2B). The ASA/DHA ratio was significantly lower (4.1) in wheat plants exposed to SO₂ for four weeks when compared to that of plants exposed to ambient or high CO₂ (8.1). However, an exposure of to high CO₂ and SO₂ in combination enhanced the ASA/DHA ratio to 17.1 (Fig. 2B).

Exposure to SO₂ and, CO₂ and SO₂ in combination enhanced the total glutathione (GSH + GSSG) content by 19 and 23%, respectively, when compared to that of plants exposed to ambient or high CO₂ (Fig. 3A). Similar to ascorbic acid, significant changes were also apparent in the redox state of glutathione of plants exposed to high SO₂ and, CO₂ and SO₂ in combination. The GSH/GSSG ratio of wheat plants exposed to high SO₂ was significantly lower (4.9) when compared to that of plants exposed to ambient or high CO₂ for four weeks (6.1). However, growth under high CO₂ and SO₂ in combination increased the GSH/GSSG ratio to 12.1 when compared to that of plants exposed to ambient or high CO₂ (Fig. 3B).

Even though the antioxidant levels and their redox states were affected by SO₂ and high CO₂, the observed results do not provide a clear picture whether the changes are the cause or consequence of altered metabolism. For instance, recently it has been demonstrated that thiol accumulation in foliar tissue is a general phenomenon when the sulfate is directly supplied to leaves by-passing the sulfate uptake by roots (De Kok 1990, De Kok & Stulen 1993, Stulen & De Kok 1993). In addition to glutathione content...
thione, substantial amounts of cysteine and γ-glutamyl-cysteine (in darkness) may accumulate upon the direct supply of sulfur to leaves, which is likely due to the lack of strict regulation of the size and composition of the thiol pool under these conditions (De Kok 1990, De Kok & Stulen 1993). Therefore, the observed accumulation of glutathione in wheat leaves may be explained by the direct assimilation of the part of deposited SO₂, rather than it has adaptive value in the protection of plants against the toxic effects of SO₂ (De Kok 1990, De Kok & Stulen 1993).

Impact of SO₂ exposure on plant ascorbate levels are rather inconsistent. In general, ascorbic acid upon SO₂ exposure may either increase (Rao & Dubey 1993), decrease or remain unchanged (Mada Manchi & Alschier 1991). Recently, Badiani & al. 1993 reported decreased levels of ascorbate in soybean growing at a natural site with strongly elevated CO₂ levels. From the present study it is evident that SO₂ exposure may result in enhanced ascorbate levels, especially upon combined exposure with high CO₂. However, the ascorbic acid remained unaltered in plants exposed to high CO₂ alone. Therefore, the physiological significance of increased levels of ascorbate upon exposure to high CO₂ and SO₂ is rather unclear.

The observed changes in the redox state of ascorbate and glutathione may be more or less the consequence of an altered metabolism. Interpretation of changes in the redox state of ascorbate and glutathione are complexed by the lack of information on the subcellular localization of accumulated ascorbate and glutathione. Although the observed decreases and increases in the redox state of ascorbate and glutathione in plants exposed to high SO₂ or high CO₂ and SO₂, in combination, appear to be related to the changes in plant sensitivity, further indepth studies are required to establish the fact.

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