Photosynthesis and photorespiration in soybean 
[Glycine max (L.) Merr.] chronically exposed

to elevated carbon dioxide and ozone

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Abstract

The effects of elevated carbon dioxide (CO2) and ozone (O3) on soybean [Glycine max (L.) Merr.] photosynthesis and photorespiration-related parameters were determined periodically during the growing season by measurements of gas exchange, photorespiratory enzyme activities and amino acid levels. Plants were treated in open-top field chambers from emergence to harvest maturity with seasonal mean concentrations of either 364 or 726 μmol mol−1 CO2 in combination with either 19 or 73 nmol mol−1 O3 (12 h daily averages). On average at growth CO2 concentrations, net photosynthesis (A) increased 56% and photorespiration decreased 36% in terminal mainstem leaves with CO2-enrichment. Net photosynthesis and photorespiration were suppressed 30% and 41%, respectively, by elevated O3 during late reproductive growth in the ambient CO2 treatment, but not in the elevated CO2 treatment. The ratio of photorespiration to A at growth CO2 was decreased 61% by elevated CO2. There was no statistically significant effect of elevated O3 on the ratio of photorespiration to A. Activities of glycine oxidase, hydroxyprolyvate reductase and catalase were decreased 10–25% by elevated CO2, and by 46–66% by elevated O3 at late reproductive growth. The treatments had no significant effect on total amino acid or glycine levels, although serine concentration was lower in the elevated CO2 and O3 treatments at several sampling dates. The inhibitory effects of elevated O3 on photorespiration-related parameters were generally commensurate with the O3-induced decline in A. The results suggest that elevated CO2 could promote productivity both through increased photoassimilation and suppressed photorespiration.

Key words: Photorespiration, CO2-enrichment, ozone, climate change, air pollution.

Introduction

The expected doubling of present-day atmospheric CO2 levels in the next century has been calculated to about halve photorespiration relative to net photosynthesis (A) in C3 plants, excluding any acclimation or temperature effects (Sharkey, 1988; Long, 1991). Thus, for most C3 plants at current temperatures, the ratio of carbon fixed by photoassimilation will increase compared with the carbon lost through photorespiration (Long, 1991). This increase in A should contribute to the productivity increase expected from atmospheric CO2 enrichment.

The air pollutant, tropospheric O3, is a potentially interacting factor with the effect of CO2 enrichment on productivity (Allen, 1990). Although damaging concentrations of O3 during the growing season tend to be regionalized, tropospheric O3 is steadily increasing on a global scale (Fishman, 1991). Net photosynthesis, growth and yield are suppressed in many plants by the levels of...
O$_3$ currently found in industrialized countries, but the effects of O$_3$ on photorespiration are less well documented (Miller, 1988).

The effects of CO$_2$ enrichment and O$_3$ on photosynthesis and photorespiration are mediated in part through a common enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Long, 1994). Carbon dioxide and O$_3$ are competitive substrates for Rubisco, and their partial pressures affect the rates of ribulose-1,5-bisphosphate (RuBP) carboxylation and oxygenation (Farquhar et al., 1980). Normally, the most significant regulator of flux through these pathways is the relative concentration of CO$_2$ and O$_3$ at the active site of Rubisco (Wallsgrove et al., 1992). This is why atmospheric CO$_2$ enrichment is expected to promote photosynthetic carbon reduction over photorespiratory carbon oxidation. Ozone, on the other hand, suppresses photosynthesis in part by decreasing Rubisco activity and content (Pell et al., 1994). Photorespiration would be expected to decline if Rubisco activity decreased, although its relative sensitivity to O$_3$ is unknown.

Photorespiration is the light-dependent release of CO$_2$ that is sensitive to the O$_2$ concentration and that originates mainly from the metabolism of compounds through the glycolate pathway (Canvin, 1979). Photorespiration begins with the oxygenation of RuBP by Rubisco to form phosphoglycolate, which is hydrolysed to glycolate by a phosphatase in the chloroplast and then excreted (Ogren, 1984). In the peroxisome, glycolate is oxidized to glyoxylate by glycolate oxidase and then transaminated to glycerate using either glutamate or serine (Ogren, 1984). Catalase decomposes the H$_2$O$_2$ formed during glycolate oxidation. Glycine enters a mitochondrion where almost all the CO$_2$ evolved in photorespiration comes from the oxidation of glycine to form serine and ammonia (Ogren, 1984). Serine then passes back to the peroxisome where it is transaminated to hydroxyproline and then reduced by hydroxypyruvate reductase to glyceric acid. Glyceric acid may be returned to the chloroplast and phosphorylated to form 3-phosphoglyceric acid, thus completing the photorespiratory carbon oxidation cycle (Ogren, 1984).

It has been suggested that decreased photorespiration due to CO$_2$ enrichment might be accompanied by decreases in the activities of photorespiratory pathway enzymes (Bowes, 1991; Webber et al., 1994). Nitrogen resources utilized in this pathway would thus be conserved. However, decreased levels of catalase, an enzyme that putatively helps protect plants against injury from O$_3$ (Declaire et al., 1984; Matters and Scandalios, 1987; Morré et al., 1990; Willekens et al., 1994), might be detrimental to plants exposed to elevated O$_3$ in combination with elevated CO$_2$ (Polle et al., 1993). The effects of O$_3$ on the photorespiratory enzymes glycolate oxidase and hydroxyproline reductase have not been reported.

Glycine and serine, the products of glycolate metabolism, accumulate in the cell, and their levels can be influenced by CO$_2$ concentration (Servaites and Ogren, 1977). Elevated CO$_2$ decreased the concentration of glycine in leaf tissues of an Arabidopsis thaliana mutant unable to convert glycine to serine (Somerville and Somerville, 1983) and lowered glycine-serine levels in excised wheat (Triticum aestivum L.) leaves (Sen Gupta, 1988), presumably due to suppressed photorespiration. In contrast, glycine and serine levels increased in leaves of kidney bean (Phaseolus vulgaris L.) after exposure to 200 nmol mol$^{-1}$ O$_3$ in controlled environments, and it was concluded that photorespiration was enhanced by exposure to O$_3$ (Ito et al., 1985). For bush bean treated in open-top chambers for 34 d with up to 110 nmol mol$^{-1}$ O$_3$, however, there were no significant changes in serine or glycine levels (Manderscheid et al., 1991).

Most of the previous studies on the effects of elevated CO$_2$ and O$_3$ on photorespiration have been short-term, controlled environment experiments. The results of such studies cannot fully reveal how photosynthesis and photorespiration in field-grown plants will respond to long-term exposure of elevated CO$_2$ and O$_3$. It is unclear which responses observed in short-term studies will occur or persist in plants grown for an entire season in elevated levels of CO$_2$ and O$_3$. Therefore, the objective of this study was to determine how photosynthesis and photorespiration-related parameters such as calculated photorespiration rates, relevant enzyme activities and amino acid levels were affected by chronic exposure to elevated levels of these gases under field conditions.

Materials and methods

Plant material and gas treatments

The experiment was performed at a site 5 km south of Raleigh, North Carolina, USA (36° N, 79° W). Soybean (cv. Essex) seeds treated with a commercial Bradyrhizobium preparation were planted on 1 June 1994 and grown to harvest maturity in 141 and 211 pots containing a 2:1:1 (by vol.) mixture of sandy loam soil:sand:Metro Mix 220 (WR Grace Co.) (pH 6.1). Plants were irrigated with drip tubes as needed to prevent water stress and fertilized biweekly with 1 l per pot of a solution containing 2.5 g l$^{-1}$ nitrogenous fertilizer (S.T.E.M., Peters Fertilizer Products). Insects and mites were controlled with applications of acephate (Orthene 75 SP at 1.7 ml l$^{-1}$), bifenthrin (Talstar F at 2.5 ml l$^{-1}$) and avermectin (Avid, 0.15 EC at 0.03 ml l$^{-1}$).

Plants were treated for 106 d in 2.4 m tall by 3 m diameter open-top field chambers (Heagle et al., 1973). Carbon dioxide gas was dispensed from a 14 ton liquid receiver 24 h daily and monitored with an infrared CO$_2$ analyser (Model 6252, Li-Cor, Inc.) (Rogers et al., 1983). Carbon dioxide treatments were ambient air (AA) or ambient air with CO$_2$ added to obtain about twice the ambient air concentration (+ CO$_2$). Ozone was produced by electrostatic discharge in dry O$_2$ (Gravitron Technics Corp.) and monitored using a UV photometric O$_3$ analyser.
Gas-exchange measurements

The Essex soybean cultivar is a determine growth variety and terminates its growth on the main stem with a terminal node bearing a trifoliate leaf. The terminal mainstem leaf was initiated within a 4 d period around 52 d after planting (DAP) in all the treatments. Net CO$_2$ assimilation rate of the terminal mainstem leaf was measured in the field periodically during the growing season between 54 and 103 DAP. In situ measurements of $A$ were made in the chambers between 10.00 and 14.00 EST using a portable photosynthesis system with a 11 cuvette (Model 6200, Li-Cor, Inc.) and Version 6.2 software. The CO$_2$ analyser was calibrated at the beginning of each measurement period. During the measurements, which were each completed within 45 s, the average PAR, leaf temperature and relative humidity were 1700 μmol m$^{-2}$ s$^{-1}$, 33 °C and 48%, respectively. Midday leaf temperatures obtained with a steady-state porometer (Model 1600, Li-Cor, Inc.) on 30 occasions (502 measurements) during the season averaged 29–30 °C among treatments.

Net CO$_2$ assimilation rate of an upper mainstem canopy leaf was also measured in the laboratory using a 3.5 l Lexan cuvette in an open gas-exchange system (Fiscus et al., 1997). The gas exchange measurements were conducted in the laboratory four times during the field season beginning at 37 DAP and concluding at 106 DAP. Leaf areas were determined non-destructively from outlines of the measured leaflets using a leaf area meter (Model 3050A, Li-Cor Inc.). Air was supplied to the chamber through two manifolds and rapidly mixed by two fans. Air flow rate was varied from 5.0 to 9.5 l min$^{-1}$ as needed to minimize CO$_2$ di effects. Flow rates were measured by mass flow meters, which were calibrated using a bubble flow meter. Temperature of the cuvette was controlled with a copper cold plate in contact with the lower surface of the chamber through which water from a temperature-controlled water bath was circulated. Two shaded copper-Constantan thermocouples were used to measure temperature in the cuvette and three thermocouples were pressed against the abaxial leaf surface to measure leaf temperature. Temperature in the cuvette was adjusted to maintain leaf temperature at a nominal 28 °C. The chamber was illuminated with four 500 W quartz halogen lamps that provided a photon flux density of 1300 μmol m$^{-2}$ s$^{-1}$ PAR. Although this PAR was lower than full sunlight, it was above light saturation for photosynthesis, as measured by the response of $A$ to PAR. Carbon dioxide and H$_2$O vapour concentrations were measured using an infrared gas analyser (Model 6262, Li-Cor, Inc.) in absolute mode. Both the CO$_2$ and H$_2$O signals were checked daily for zero and the appropriate span concentration. The desired CO$_2$ concentration was obtained by mixing CO$_2$-free air from a Balston compressed air purifier and dryer (Model 75–62 FT-RT) with 5% CO$_2$ in compressed air using mass flow controllers. The mixed air was humidified by saturating a fraction of the air with water vapour to maintain a vapour pressure deficit of 1.5–1.6 kPa (50–55% relative humidity) in the cuvette.

On each of six evenings prior to the gas exchange measurements, four plants (one per treatment) were transported to the laboratory and left overnight in a darkened room at 22 °C. The following morning, an upper mainstem leaf was sealed in the cuvette and allowed to equilibrate at growth CO$_2$ concentrations, measurement light levels and humidity. Measurements of $A$ were made first at growth CO$_2$ concentration ($A_{CO_2}$) and then in regular CO$_2$ concentration steps from 60 μmol mol$^{-1}$ to growth CO$_2$ concentrations. At each interval, $A$ was measured after steady-state conditions were reached (5–10 min). The measurements at low CO$_2$ concentrations were thought to have little permanent effect on Rubisco activity because, in 92% of the curves, both initial and final $A_{CO_2}$ measurements were within the 95% confidence interval for values predicted by an empirical model of $A/C_i$.

The CO$_2$ compensation point ($I'$) and the initial slope at $I'$ (carboxylation efficiency, $CE$) were calculated from an exponential model fitted to the gas exchange data (Jacob et al., 1995; Reid and Fiscus, unpublished results). The maximum Rubisco carboxylation velocity ($v_{max}$) and the mitochondrial respiration ($R_i$) were then calculated according to Farquhar et al. (1980) and von Caemmerer and Farquhar (1981) using $I'$, $CE$, Rubisco Michaelis constants for soybean (Reid and Fiscus, unpublished results), and a $F^*$ value of 5.02 Pa, which included a correction for the temperature effect at 28 °C (Brooks and Farquhar, 1985).

The rate of photorespiration at growth CO$_2$ levels was calculated as described by Farquhar et al. (1980) and Sharkey (1988):

$$\text{Photorespiration} = 0.5n_0 = 0.5 \left[ \frac{A_{CO_2} + R_i}{C_i - 27^\circ - 0.5} \right]$$

The CO$_2$ concentrations used for the calculations are the intercellular concentrations ($C_i$).

Enzyme assays

For the enzyme and amino acid assays of leaf tissues during vegetative growth (27 DAP), a fully expanded leaf exposed for 1 week to the treatments was sampled. For assays of leaf tissues during reproductive growth (≥54 DAP), a leaflet from the terminal leaf or first mainstem leaf below the terminal leaf was taken from two plants grown in 14 l pots in each of eight additional chambers that were part of a growth and yield experiment (two replicates per treatment). Leaflets obtained from two plants in each chamber were combined into a single sample for analysis.

Fresh leaf tissue (0.5 g) samples were ground in a chilled mortar with 25 mg PVPP and 3 ml of 50 mM TRIS–HCl buffer (pH 7.8) containing 0.01% (v/v) Triton X-100 and 5 mM dithiothreitol. The homogenate was centrifuged at 30 000 g for 20 min at 4 °C. The supernatant was decanted and immediately used for enzyme and total free amino acid assays. Aliquots were frozen at −80 °C for later analysis of glycine and serine levels. Recoveries were assumed to be the same among treatments.

Glycated oxidase (EC 1.1.3.1) was assayed as described by Feierabend and Beever (1972), with modifications. A 2 ml volume of assay mixture contained 50 mM TRIS–HCl buffer (pH 7.8), 0.009% (v/v) Triton X-100, 3.3 mM phenylhydrazine HCl (pH 6.8), 50 μl of plant extract, and 5 mM glycic acid (neutralized to pH 7.0 with KOH) to start the reaction. Glycolate oxidase activity was determined by following the formation of glyoxylate phenylhydrazone (extinction coefficient
of 17 mM⁻¹ cm⁻¹ at 324 nm for 2 min after an initial lag phase of 1 min.

Hydroxypyruvate reductase (EC 1.1.1.29) was assayed as described by Schützegabel and Siegenthaler (1984), with modifications. A 2 ml volume of assay mixture contained 50 mM TRIS–HCl buffer (pH 7.8), 2 mM hydroxypyruvate, 200 μM NADH, and 50 μl of plant extract to start the reaction. Enzyme activity was determined by following the oxidation of NADH by fluorescence (excitation c 615 nm, emission c 520 nm) at 340 nm for 30 s.

Catalase (EC 1.11.1.6) was assayed according to Aebi (1983). The 2 ml of reaction mixture contained 50 mM TRIS–HCl buffer (pH 7.0), 10 mM H₂O₂, and 10 μl of plant extract to start the reaction. Catalase activity was determined by following the decomposition of H₂O₂ (excitation c 400 nm, emission c 520 nm) at 240 nm for 1 min.

In each enzyme assay, a blank reaction was run for each sample without substrate. All assays were run at 25 °C in a temperature-controlled cuvette.

**Amino acid assays**

Plant extract from each replicate was diluted 1:10 with water, mixed with an equal volume of 10% (w/v) trichloroacetic acid, incubated for 30 min at 4 °C, and centrifuged at 16000 g for 5 min. A 0.5 ml aliquot of each supernatant was adjusted to pH 5.0 with 2 N KOH and treated with ninhydrin solution (Plummer, 1971). Total free amino acid concentration was determined colorimetrically by absorbance at 570 nm. Glutamate was used to construct a standard curve, and results were expressed as glutamate equivalents.

The determination of glycine and serine by HPLC was initiated by mixing 100 μl of plant extract with 4.3 ml of 4% (w/v) sulphasalicylic acid containing β-DL-(2-thienyl)alanine as an internal standard. After incubation on ice for 1 h and centrifugation at 20000 g for 15 min, the supernatant was neutralized with 1 N NaOH and diluted 1:10 with 100 mM borate buffer (pH 8.5). For precolumn derivatization, 30 μl of the plant extract-borate buffer solution was mixed with 30 μl 9-fluorenylmethyl chloroformate (FMOC-Cl) in acetone and, after 4 min, extracted with 90 μl pentane:ethylacetate (80:20, v/v) (Emarsen et al., 1983). An aliquot of the lower phase containing the FMOC-amino acids was fractionated by reversed-phase gradient HPLC using a Varian AminoTag amino acid analysis column as described by Manderscheid et al. (1991). Quantification was done by integration of the fluorescence chromatogram (excitation 260 nm, emission 310 nm) with a Pye Unicam data system. A standard amino acid mixture (Sigma Chemical Co.) was used to calibrate the analysis.

**Statistics**

For the gas exchange measurements, three plants per chamber were measured in the field and in the laboratory at each sampling period. Results from field and laboratory measurements were averaged separately for use as treatment replicates. There were two replicate chambers for each of the four treatments. For the enzyme and amino acid assays, there were four replicate chambers for each of the four treatments.

Treatments were assigned to chambers using a completely randomized factorial. Sampling period was treated as a split-plot treatment within the whole-plot units of chambers. Thus, the results were analysed as a split-plot design, with O₃ and CO₂ as the whole-plot treatments and sampling period as the split-plot treatment. If a significant sampling period by main effect interaction was detected, data were analysed separately for each sampling period using a completely randomized factorial model. If a significant main effect interaction was detected within a sampling period, pairwise comparisons were made between treatments to identify significant differences. Data were tested for homogeneity of variance and normality prior to analysis (Sokal and Rohlf, 1981). Net CO₂ assimilation rate, photorespiration rate, the ratio of photorespiration rate to Aₐ/Cᵢ, and amino acid data were log transformed prior to analysis (Sokal and Rohlf, 1981). 95% confidence limits were calculated for the retransformed data. Statistical tests were considered significant if P ≤ 0.05 and marginally significant if 0.05 < P ≤ 0.10.

**Results**

**CO₂ and O₃ concentrations, and environmental conditions**

Daily 12 h average (+sd) CO₂ concentration in the AA and +CO₂ treatments was 364 ± 12 and 726 ± 80 μmol mol⁻¹, respectively (Table 1). The daily 12 h average ambient O₃ concentration was 48 ± 13 nmol mol⁻¹ for the 106 d experiment (Table 1). Daily 12 h average O₃ concentration in the CF and +O₃ treatments was 19 ± 7 and 73 ± 23 nmol mol⁻¹, respectively (Table 1). Additional environmental parameters measured during the experiment also are shown in Table 1.

<table>
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<tr>
<th>Parameter</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>Season</th>
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</thead>
<tbody>
<tr>
<td>Average daily CO₂ conc. (μmol mol⁻¹)²</td>
<td>360</td>
<td>364</td>
<td>361</td>
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<td>364</td>
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<td>+CO₂</td>
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<td>691</td>
<td>766</td>
<td>747</td>
<td>726</td>
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<tr>
<td>Average daily O₃ conc. (nmol mol⁻¹)³</td>
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<td>44</td>
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<tr>
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<td>15</td>
<td>16</td>
<td>19</td>
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<tr>
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<td>63</td>
<td>73</td>
<td>77</td>
<td>73</td>
</tr>
<tr>
<td>SUM06 (ppm h⁻¹)²</td>
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<td>4.2</td>
<td>5.9</td>
<td>5.0</td>
<td>23.3</td>
</tr>
<tr>
<td>CF</td>
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<td>0.4</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>O₃</td>
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<td>20.8</td>
<td>12.9</td>
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<td>30</td>
<td>26</td>
<td>29</td>
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<td>22</td>
<td>19</td>
<td>15</td>
<td>19</td>
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<tr>
<td>Minimum temperature (°C)</td>
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<td>66</td>
<td>62</td>
<td>66</td>
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<td>35</td>
<td>37</td>
<td>30</td>
<td>36</td>
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</table>

²Daily 12 h (08.00–20.00 h EST) CO₂ concentrations in ambient CO₂ open-top chambers (AA) and in the elevated CO₂ open-top chambers (+CO₂). Carbon dioxide gas was added to ambient air 24 h daily to obtain about twice the ambient air concentration.

³Daily 12 h (08.00–20.00 h EST) average O₃ concentrations in ambient air (Ambient), in charcoal-filtered air open-top chambers (CF), and in non-filtered air with added O₃ open-top chambers (+O₃). Ozone was added to non-filtered air 12 h daily (08.00–20.00 h EST) at 1.5 times the ambient air concentration.

⁴SUM06 is the sum of all hourly average O₃ concentrations ≥ 60 nmol mol⁻¹.
Gas exchange measurements

The rates of gas exchange measured in the field showed that $A_{CO_2}$ was significantly increased an average 56% in the +$CO_2$ treatments (Fig. 1; Table 2). Average $A_{CO_2}$ was suppressed 30% in the +$O_3$-$AA$ treatment at the 75–83 and 97–106 DAP sampling periods, but not in the +$O_3$ + $CO_2$ treatment (Fig. 1; Table 2). Similar treatment effects were seen in gas-exchange measurements of $A_{CO_2}$ made in the laboratory (data not shown).

Calculated photorespiration rates were significantly decreased an average 36% in the +$CO_2$ treatments (Fig. 2; Table 2). Photorespiration rates were suppressed 12% in the +$O_3$ treatments at the 54–62 DAP sampling period (Fig. 2; Table 2). However, the inhibitory effect of elevated $O_3$ did not persist in the +$O_3$ + $CO_2$ treatment. At the 75–83 DAP and 97–106 DAP sampling periods, the average photorespiration rate was 41% lower in the +$O_3$-$AA$ treatment compared with the CF-$AA$ treatment, but there was no statistically significant effect of elevated $O_3$ in the +$CO_2$ treatments (Fig. 2; Table 2).

On an absolute basis, photorespiration rates in the CF-$AA$ treatment averaged 38 ± 1% (± se) of $A_{CO_2}$ among sampling periods in laboratory-based measurements. In the CF + $CO_2$ treatment, photorespiration rates averaged 14 ± 1% of $A_{CO_2}$ among sampling periods. Relative to the photorespiration rate in the CF-$AA$ treatment, the photorespiration rate in the CF + $CO_2$ treatment was 63% lower ($P<0.001$). There was no statistically significant effect of elevated $O_3$ on the ratio of photorespiration to $A$ ($P>0.2$).

The average $C_i$ of plants measured in the laboratory at growth $CO_2$ levels changed from 219 ± 20 $\mu$mol $CO_2$ in the AA treatments to 477 ± 39 $\mu$mol $CO_2$ in the +$CO_2$ treatments among sampling periods (Table 2). During the last sampling period, the average $C_i$ in the +$O_3$-$AA$ treatment (259 ± 7 $\mu$mol $CO_2$) was 25% higher than that in the CF-$AA$ treatment (207 ± 13 $\mu$mol $CO_2$) (Table 2). Field measurements of $A_{CO_2}$ showed that $C_i$ also about doubled in the +$CO_2$ treatments ($P<0.01$). However, in contrast to the laboratory measurements, field measurements of plants in the +$O_3$-$AA$ treatment showed that $C_i$ was not significantly different from that in plants in the CF-$AA$ treatment ($P<0.5$).

Photorespiratory cycle enzymes

Activities of glycolate oxidase, hydroxypyruvate reductase and catalase were 10–25% lower on a fresh weight basis in the +$CO_2$ treatments at the 27 and 77 DAP sampling periods compared with the AA treatments (Fig. 3; Table 3). Glycolate oxidase activity was particularly suppressed in the CF + $CO_2$ treatment at 27 DAP. Glycolate oxidase and catalase activities were lower in the +$CO_2$ treatments at 91 DAP, but the differences were only marginally significant. Glycolate oxidase activity was slightly higher in the +$O_3$ treatments at 27 DAP. Otherwise, activities of these enzymes generally were not significantly different in the +$O_3$ treatments compared with the CF treatments, except at the 105 DAP sampling period when activities were 46–66% lower in the +$O_3$-$AA$ treatment compared with the CF-$AA$ treatment (Fig. 3; Table 3). However, the inhibitory effect of elevated $O_3$ on enzyme activity was not statistically significant when plants were treated concurrently with elevated $CO_2$ (Fig. 3; Table 3).

Amino acids

Total free amino acid equivalents and glycine levels among treatments increased from an average of 18 ± 1...
and $0.9 \pm 0.1 \mu mol \cdot m^{-2} \cdot h^{-1}$, respectively, at 27 DAP to $27 \pm 1$ and $1.3 \pm 0.2 \mu mol \cdot m^{-2} \cdot h^{-1}$, respectively, at 91 DAP. Levels decreased at 105 DAP to $22 \pm 1$ and $0.8 \pm 0.1 \mu mol \cdot m^{-2} \cdot h^{-1}$ for total amino acid equivalents and glycine, respectively. There were no significant differences among treatments (Table 3). Average serine levels were significantly lower in the +CO$_2$ treatments at the 27 DAP and 77 DAP sampling periods compared with the AA treatments (Fig. 3d; Table 3). Ozone effects on serine levels were statistically significant only at the 105 DAP sampling period when average serine levels were lower in the +O$_3$ treatments compared with the CF treatments (Fig. 3d; Table 3).

### Discussion

#### Effects of elevated CO$_2$

Our calculations supported the prediction that a doubling of CO$_2$ would about halve photosynthesis relative to $A$ (Sharkey, 1988; Long, 1991). The average photosynthesis rate in the CF+CO$_2$ treatment was 63% lower than that in the CF-AA treatment. The increase in $C_i$ in the +CO$_2$ treatments was probably the primary cause of this response (Ogren, 1984; Wallsgrove et al., 1992). Increased $C_i$ could suppress RuBP oxygenation and increase RuBP carboxylation, which in turn would lead to increased $A_{CO_2}$ in the +CO$_2$ treatments (Fig. 1). The decreases in $A_{CO_2}$ at the 97–106 DAP sampling period compared with previous measurements were likely developmentally related, although photosynthesis was still suppressed by elevated CO$_2$ (Fig. 2).

Previous experiments have not always found that elevated CO$_2$ suppressed photosynthesis. For example, in a controlled environment study with wheat, photosynthesis rate relative to $A_{CO_2}$ was decreased 55% in 1200 $\mu mol \cdot mol^{-1}$ CO$_2$ compared with ambient CO$_2$, but the absolute rate of photosynthesis as measured by the ratio of $14CO_2$ to $12CO_2$ uptake was not significantly different between treatments (Kendall et al., 1985). In the past, discrepancies between our understanding of photosynthesis and experimental data have usually been attributed to inadequacies in the methods used to measure photosynthesis (Zelitch, 1979; Somervile and Somervile, 1983; Sharkey, 1988), and perhaps the same is true in this case. Using the biochemical model of photosynthesis (Farquhar et al., 1980) and Rubisco kinetics (Laing et al., 1974), Besford et al. (1985) calculated that the rate of photosynthesis in tomato (Lycopersicon esculentum L.) was suppressed 53% in 1200 $\mu mol \cdot mol^{-1}$ CO$_2$ compared with 300 $\mu mol \cdot mol^{-1}$ CO$_2$. Recently, Kent et al. (1992) extended this approach to include O$_3$ uptake in the light using mass spectrometry measurements with $^{18}O_2$. The method was used in a controlled environment study with lilac (Syringa vulgaris L.) and sunflower (Helianthus annuus L.) to show that the absolute rate of photosynthesis and the rate relative to $A_{CO_2}$ were lowered by 43% and by 57–70%, respectively, in 682 $\mu mol \cdot mol^{-1}$ CO$_2$ compared with 344 $\mu mol \cdot mol^{-1}$ CO$_2$.

Our results indicated that the suppression of photosynthesis by elevated CO$_2$ was sustained over most of the growing season in soybean, and thus might contribute to increased growth and yield. The suppression of photosynthesis rates by CO$_2$ enrichment was accompanied by relatively small decreases in photosynthetic enzyme activities (Fig. 3). These results partially support the proposition by Bowes (1991) and Webber et al. (1994) that CO$_2$ enrichment might conserve nitrogen in the photosynthetic cycle enzymes. It has been suggested...
Effects of elevated CO₂

that, probably, there is no direct metabolic regulation of gene expression for photosynthetic cycle enzymes in response to CO₂, although indirect effects of CO₂-enrichment on plant development could influence enzyme levels (Walls et al., 1992). Previous short-term studies that used high levels of CO₂ to suppress photorespiration have reported decreases of 33–50% in glycolate oxidase activity on a fresh weight basis for barley (Hordeum vulgare L.) (Fair et al., 1973) and tomato (Hicklenton and Jollie, 1980). Catalase activity in tobacco (Nicotiana sp.) leaves decreased 50% after plants were transferred from air to 1000 μmol mol⁻¹ CO₂, but the activities of glycolate oxidase and hydroxypyruvate reductase were unchanged (Havir and McHale, 1989). No effect was found on glycolate oxidase mRNA abundance in tomato leaves following exposure to 2000 μmol mol⁻¹ CO₂ for 9 d (Van Oosten et al., 1994). However, accumulation of hydroxypyruvate reductase mRNA in cotyledons of dark-adapted cucumber (Cucumis sativus L.) was inhibited 20–50% during a 4 h white light irradiation in 700 μmol mol⁻¹ CO₂ compared with accumulation in 350 μmol mol⁻¹ CO₂ (Bertoni and Becker, 1996). In 1-year-old needles of spruce (Picea abies L. Karst) trees that had been treated in open-top chambers for two years, activities on a unit protein basis of glycolate oxidase and hydroxypyruvate reductase were 17–38% lower when treated with 480 and 570 μmol mol⁻¹ CO₂ than with ambient CO₂ (Van Oosten et al., 1992). Catalase activity was decreased in both year-classes of needles from spruce trees treated for six months in open-top chambers with 480 and 570 μmol mol⁻¹ CO₂, and concurrent exposure to 80 nmol mol⁻¹ O₃ exacerbated this effect in the current year’s needles (Polle et al., 1993).

Lower levels of the photorespiration metabolite, serine, in the +CO₂ treatments suggested that photorespiration was decreased by CO₂ enrichment (Fig. 3d). However, it was unclear why only serine and not glycine levels were lower in the +CO₂ treatments if suppressed photorespiration was the primary controlling factor. Other mechanisms for serine and glycine biosynthesis likely exist (Somerville and Somerville, 1983), which, along with variability in pool sizes and sink demands, could negate the inhibitory effects of elevated CO₂ on glycine-serine accumulation.

Effects of elevated O₃

The O₃ portion of the experiment indicated that A₉ CO₂, photorespiration rates, photorespiratory enzyme activities and serine levels eventually decreased in response to elevated O₃. At the 97–106 DAP sampling period, increased Ci levels in the O₃ treatments were insufficient to account for the extent of the depression in photorespiration. Previous studies of catalase found that enzyme activities and mRNA levels either were unchanged (Matters and Scandalios, 1987; Sharma and Davis, 1994; Rao et al., 1996), increased (Declaire et al., 1984; Willekens et al., 1994) or decreased (Declaire et al., 1984) in leaves following exposure to O₃. Morré et al. (1990) reported that catalase activity in soluble extracts from O₃-treated spruce needles was similar to that in the controls, but that activity in the particulate fraction of the extract increased as well as the number of peroxisomes. Catalase activity in spruce needles decreased following exposure to elevated CO₂ and O₃ (Polle et al., 1993). In this study, photorespiratory enzyme activities and serine levels declined along with A₉ CO₂ and calculated
Table 3. Analysis of variance results (P value) for enzyme and amino acid assays at five sampling periods from 27 to 105 DAP

At each sampling period, glycolate oxidase (Glyox), hydroxypyruvate reductase (Hydrd), catalase (Cat) and total free amino acids (AA) were assayed in fresh extracts of canopy leaves from four replicates of each treatment combination. Frozen extracts were later assayed for glycine (Gly) and serine (Ser) concentrations. Enzyme activities and amino acid levels were expressed on a fresh weight basis. Analysis of variance results overall (A) and by sampling period (B) are shown.

<table>
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<tr>
<th>Source</th>
<th>df</th>
<th>Glyox</th>
<th>Hydrd</th>
<th>Cat</th>
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<th>Gly</th>
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<td>0.001</td>
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<td>0.14</td>
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*Pairwise comparison of CF-AA versus CF-$CO_2$ indicated significant difference ($P=0.05$).

*Pairwise comparison of CF-AA versus $O_3$-AA indicated significant differences ($P<0.001$); pairwise comparison of CF+$CO_2$ versus $O_3$+$CO_2$ indicated no significant differences ($P<0.4$).

rates of photorespiration, which was attributed to the general metabolic injury caused by chronic exposure to $O_3$.

By 105 DAP, plants showed severe foliar injury in the +$O_3$-AA treatment. There was no indication that photorespiration was stimulated by $O_3$.

**Effects of $CO_2$ and $O_3$ combined**

Elevated $CO_2$ generally suppressed the detrimental effects of elevated $O_3$. Several previous studies have reported that elevated $CO_2$ alleviated the inhibitory effects of $O_3$ on $A$ (Barnes and Pfirrmann, 1992; Mulchi et al., 1992; Balaguer et al., 1995; McKee et al., 1995). Decreased stomatal conductance induced by elevated $CO_2$ would reduce $O_3$ uptake and hence injury [Allen, 1990; McKee

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Fig. 3. Effects of elevated $CO_2$ and $O_3$ on the activity of glycolate oxidase (a), hydroxypyruvate reductase (b), catalase (c), and the level of serine (d) in mainstem leaves during five sampling periods from 27–105 DAP. Treatments as in Fig. 1. Values are means ± 95% confidence intervals.
et al., 1995; Fiscus et al., 1997]. It has also been suggested that increased carbohydrate availability (Allen, 1990) or enhanced antioxidant metabolism (Rao et al., 1995) arising from CO₂ enrichment were important in counteracting O₃ toxicity. If so, these results indicated that elevated catalase levels were not required for this response.

Conclusions

Elevated CO₂ could promote productivity of C₃ plants partly through increased photoassimilation and suppressed photorespiration. In our experiment with soybean, CO₂ enrichment increased A and suppressed calculated photorespiration rates during both vegetative and reproductive growth. However, the moderate effect of elevated CO₂ on photosynthetic enzyme activities and glycine/serine levels did not suggest that significant levels of nitrogen were being conserved by downregulation of these pathway components. The inhibitory effects of elevated O₃ on photosynthetic generally coincided with O₂-induced declines in ACO₂. Photorespiration-related parameters were not especially sensitive to elevated O₃. Carbon dioxide-enrichment generally diminished the determinantal effects of elevated O₃ on A and photorespiration-related parameters.

Acknowledgements

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References
