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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 (CO_2) and ozone (O_3) 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} CO_2 in combination with either 19 or 73 nmol mol^{-1} O_3 (12 h daily averages). On average at growth CO_2 concentrations, net photosynthesis *(A)* increased 56% and photorespiration decreased 36% in terminal mainstem leaves with CO_2-enrichment. Net photosynthesis and photorespiration were suppressed 30% and 41%, respectively, by elevated O_3 during late reproductive growth in the ambient CO_2 treatment, but not in the elevated CO_2 treatment. The ratio of photorespiration to *A* at growth CO_2 was decreased 61% by elevated CO_2. There was no statistically significant effect of elevated O_3 on the ratio of photorespiration to *A*. Activities of glycolate oxidase, hydroxypyruvate reductase and catalase were decreased 10–25% by elevated CO_2, and by 46–66% by elevated O_3 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 CO_2 and O_3 treatments at several sampling dates. The inhibitory effects of elevated O_3 on photorespiration-related parameters were generally commensurate with the O_3-induced decline in *A*. The results suggest that elevated CO_2 could promote productivity both through increased photoassimilation and suppressed photorespiration.

Key words: Photorespiration, CO_2-enrichment, ozone, climate change, air pollution.

Introduction

The expected doubling of present-day atmospheric CO_2 levels in the next century has been calculated to about halve photorespiration relative to net photosynthesis *(A)* in C_3 plants, excluding any acclimation or temperature e ects (Sharkey, 1988; Long, 1991). Thus, for most C_3 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 CO_2 enrichment.

The air pollutant, tropospheric O_3, is a potentially interacting factor with the e ect of CO_2 enrichment on productivity (Allen, 1990). Although damaging concentrations of O_3 during the growing season tend to be regionalized, tropospheric O_3 is steadily increasing on a global scale (Fishman, 1991). Net photosynthesis, growth and yield are suppressed in many plants by the levels of

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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_2 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_2 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 glycine using either glutamate or serine (Ogren, 1984). Catalase decomposes the H_2O_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 hydroxypyruvate and 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 (Decleire *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 hydroxypyruvate 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 14 l and 21 l 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} 10 : 30 : 20 N : P : K soluble fertilizer (Peters Fertilizer Products, WR Grace Co.). The initial fertilization included 0.31 g l^{-1} of a micronutrient formulation (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 (Griffin Technics Corp.) and monitored using a UV photometric O_3 analyser

(Model 49, Thermo Environmental Instruments Co.). Ozone treatments were charcoal-filtered air (CF) or non-filtered air with O_3 added to 1.5 times the ambient O_3 concentrations for 12 h (08.00–20.00 EST) daily ($+O_3$). The air 10 cm below the plant canopy was sampled in each chamber through Teflon tubing, and the concentration of CO_2 and O_3 in it was measured every 30 min throughout the experiment.

Gas-exchange measurements

The Essex soybean cultivar is a determinate growth variety and terminates its growth on the main stem with a terminal node bearing a trifoliolate 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 1 l 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 5.5 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 differentials. 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_2O vapour concentrations were measured using an infrared gas analyser (Model 6262, Li-Cor, Inc.) in absolute mode. Both the CO_2 and H_2O 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 (Γ) and the initial slope at Γ (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_{cmax}) and the mitochondrial respiration (R_d) were then calculated according to Farquhar *et al.* (1980) and von Caemmerer and Farquhar (1981) using Γ, *CE*, Rubisco Michaelis constants for soybean (Reid and Fiscus, unpublished results), and a Γ^* 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):

$$\text{Photorespiration} = 0.5v_0 = 0.5\left[\frac{A_{CO_2}+R_d}{\frac{C_i}{2\Gamma^*}-0.5}\right]$$

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 ($\geq$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 the chambers used for gas-exchange measurements. Similarly positioned leaflets also were obtained 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.

Glycolate oxidase (EC 1.1.3.1) was assayed as described by Feierabend and Beevers (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 glycolic 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 Schwitzguebel and Siegenthaler (1984), with modifications. A 2 ml volume of assay mixture contained 50 mM TRIS–HCl bu er (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 (extinction coe cient of 6.2 mM^{-1} cm^{-1}) 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 bu er (pH 7.0), 10 mM H_2O_2, and 10 μl of plant extract to start the reaction. Catalase activity was determined by following the decomposition of H_2O_2 (extinction coe cient of 40 mM^{-1} cm^{-1}) 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 16 000 *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) sulphosalicylic acid containing β-DL-(2-thienyl)alanine as an internal standard. After incubation on ice for 1 h and centrifugation at 20 000 *g* for 15 min, the supernatant was neutralized with 1 N NaOH and diluted 1 : 10 with 100 mM borate bu er (pH 8.5). For precolumn derivatization, 30 μl of the plant extract-borate bu er 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) (Einarsson *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 O_3 and CO_2 as the whole-plot treatments and sampling period as the split-plot treatment. If a significant sampling period by main e ect interaction was detected, data were analysed separately for each sampling period using a completely randomized factorial model. If a significant main e ect interaction was detected within a sampling period, pairwise comparisons were made between treatments to identify significant di erences. Data were tested for homogeneity of variance and normality prior to analysis (Sokal and Rohlf, 1981). Net CO_2 assimilation rate, photorespiration rate, the ratio of photorespiration rate to A_{CO_2}, C_i 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 $P \leq 0.05$ and marginally significant if $P \leq 0.10$.

Results

CO_2 and O_3 concentrations, and environmental conditions

Daily 12 h average ($\pm$sd) CO_2 concentration in the AA and $+CO_2$ treatments was 364 ± 12 and 726 ± 80 μmol mol^{-1}, respectively (Table 1). The daily 12 h average ambient O_3 concentration was 48 ± 13 nmol mol^{-1} for the 106 d experiment (Table 1). Daily 12 h average O_3 concentration in the CF and $+O_3$ 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 1. *Mean monthly and seasonal CO_2 and O_3 concentrations, cumulative, peak-weighted O_3 index (SUM06), mean daily maximum and minimum temperatures, mean relative humidity (RH), and mean daily* PAR *from 9 June through 15 September 1994*

Parameter	June	July	August	September	Season
Average daily CO_2 conc. (μmol mol^{-1})[a]					
AA	360	364	361	373	364
$+CO_2$	702	691	766	747	726
Average daily O_3 conc. (nmol mol^{-1})[b]					
Ambient	58	43	44	49	49
CF	28	20	15	16	19
$+O_3$	83	63	73	77	73
SUM06 (ppm h^{-1})[c]					
Ambient	8.2	4.2	5.9	5.0	23.3
CF	0.2	0.1	0.4	0.0	0.7
O_3	16.5	13.9	20.8	12.9	64.1
Maximum temperature (°C)	30	31	30	26	29
Minimum temperature (°C)	20	22	19	15	19
RH (%) (10.00–14.00 h EST)	67	68	66	62	66
PAR (mol m^{-2} d^{-1})[d]	41	35	37	30	36

[a]Daily 12 h (08.00–20.00 h EST) CO_2 concentrations in ambient CO_2 open-top chambers (AA) and in the elevated CO_2 open-top chambers ($+CO_2$). Carbon dioxide gas was added to ambient air 24 h daily to obtain about twice the ambient air concentration.

[b]Daily 12 h (08.00–20.00 h EST) average O_3 concentrations in ambient air (Ambient), in charcoal-filtered air open-top chambers (CF), and in non-filtered air with added O_3 open-top chambers ($+O_3$). Ozone was added to non-filtered air 12 h daily (08.00–20.00 h EST) at 1.5 times the ambient air concentration.

[c]SUM06 is the sum of all hourly average O_3 concentrations ≥ 60 nmol mol^{-1}.

[d]*PAR* data collection began on 20 June and ended on 12 September.

Gas exchange measurements

The rates of gas exchange measured in the field showed that A_{CO2} 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 e ects were seen in gas-exchange measurements of A_{CO2} 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 e ect 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 e ect 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 $38\pm1\%$ ($\pm$se) of A_{CO_2} among sampling periods in laboratory-based measurements. In the $CF+CO_2$ treatment, photorespiration rates averaged $14\pm1\%$ 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 e ect 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 μmol CO_2 in the AA treatments to 477 ± 39 μmol 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 μmol CO_2) was 25% higher than that in the CF-AA treatment (207 ± 13 μmol 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 di erent 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 di erences 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 di erent 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 e ect 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

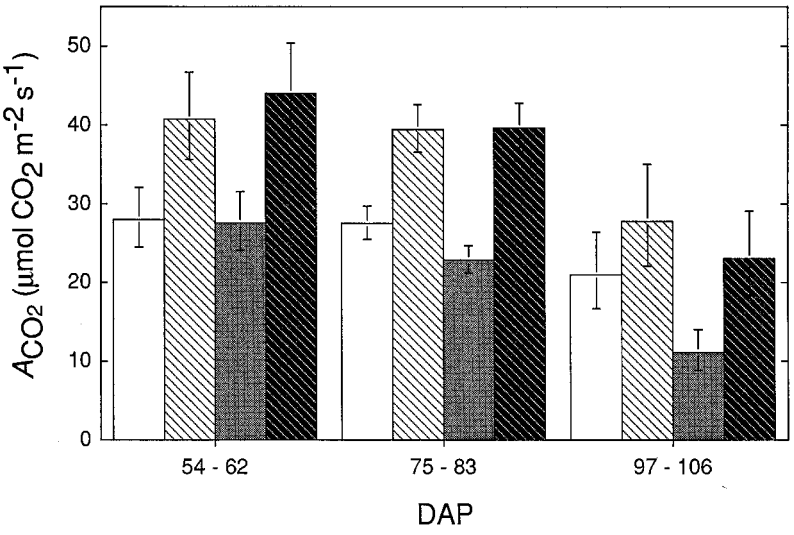

Fig. 1. E ects of elevated CO_2 and O_3 on net CO_2 assimilation rate at growth CO_2 levels (A_{CO_2}). Midday *in situ* A_{CO_2} of the terminal mainstem leaf was measured during three sampling periods from 54–106 d after planting (DAP). Plants were treated with charcoal-filtered (CF) air and ambient CO_2 □; CF air and twice ambient CO_2 ⍁; 1.5 times ambient O_3 and ambient CO_2 ▨; or 1.5 times ambient O_3 and twice ambient CO_2 ▩. Values are means $\pm$95% confidence intervals.

Table 2. *Analysis of variance results (*P *values) for midday* in situ *net CO_2 assimilation rates at growth CO_2 levels (A_{CO_2}) during three sampling periods (SP) from 54–106 d after planting (DAP)*

Analysis of variance results also are shown for photorespiration rates (*PR*) and intercellular CO_2 concentration (C_i) calculated from A/C_i curves made on plants treated in open-top field chambers and then transported to the laboratory for gas-exchange measurements during four sampling periods from 36–106 DAP. Analysis of variance results overall (A) and by sampling period (B) are shown. Treatments were ambient and twice ambient CO_2 (CO_2) in combination with CF air and supplemental O_3 (O_3); nd, not determined.

Source	df	A_{CO_2}	*PR*	C_i
(A) Overall ANOVA results				
CO_2	1	0.001	0.001	0.001
O_3	1	0.005	0.001	0.08
$CO_2 \times O_3$	1	0.016	0.001	0.06
SP	3	0.001	0.001	0.01
$SP \times CO_2$	3	0.61	0.60	0.17
$SP \times O_3$	3	0.001	0.001	0.18
$SP \times CO_2 \times O_3$	3	0.37	0.001	0.31
(B) ANOVA results by sampling period				
36–44 DAP				
CO_2	1	nd	0.001	0.001
O_3	1	nd	0.27	0.30
$CO_2 \times O_3$	1	nd	0.50	0.48
54–62 DAP				
CO_2	1	0.001	0.001	0.001
O_3	1	0.63	0.04	0.40
$CO_2 \times O_3$	1	0.47	0.29	0.44
75–83 DAP				
CO_2	1	0.001	0.004	0.001
O_3	1	0.02	0.37	0.92
$CO_2 \times O_3$	1	0.02[a]	0.04[b]	0.23
97–106 DAP				
CO_2	1	0.001	0.002	0.001
O_3	1	0.002	0.001	0.03
$CO_2 \times O_3$	1	0.06[a]	0.003[b]	0.10[c]

[a]Pairwise comparison of CF-AA versus O_3-AA indicated a significant di erence ($P<0.01$); pairwise comparison of $CF+CO_2$ versus O_3+CO_2 indicated no significant di erence ($P>0.2$).

[b]Pairwise comparison of CF-AA versus O_3-AA indicated a significant di erence ($P\leq0.05$); pairwise comparison of $CF+CO_2$ versus O_3+CO_2 indicated no significant di erence ($P\geq0.12$).

[c]Pairwise comparison of CF-AA versus O_3-AA indicated a significant di erence ($P<0.02$); pairwise comparison of $CF+CO_2$ versus O_3+CO_2 indicated no significant di erence ($P>0.4$).

and $0.9\pm0.1\ \mu mol\ g^{-1}$ FW, respectively, at 27 DAP to 27 ± 1 and $1.3\pm0.2\ \mu mol\ g^{-1}$ FW, respectively, at 91 DAP. Levels decreased at 105 DAP to 22 ± 1 and $0.8\pm0.1\ \mu mol\ g^{-1}$ FW for total amino acid equivalents and glycine, respectively. There were no significant di erences 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 e ects 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 photorespiration relative to *A* (Sharkey, 1988; Long, 1991). The average photorespiration 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_{CO2} 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 photorespiration was still suppressed by elevated CO_2 (Fig. 2).

Previous experiments have not always found that elevated CO_2 suppressed photorespiration. For example, in a controlled environment study with wheat, photorespiration rate relative to A_{CO_2} was decreased 55% in 1200 μmol mol^{-1} CO_2 compared with ambient CO_2, but the absolute rate of photorespiration as measured by the ratio of $^{14}CO_2$ to $^{12}CO_2$ uptake was not significantly di erent between treatments (Kendall *et al.*, 1985). In the past, discrepancies between our understanding of photorespiration and experimental data have usually been attributed to inadequacies in the methods used to measure photorespiration (Zelitch, 1979; Somerville and Somerville, 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 photorespiration in tomato (*Lycopersicon esculentum* L.) was suppressed 53% in 1200 μmol mol^{-1} CO_2 compared with 300 μmol mol^{-1} CO_2. Recently, Kent *et al.* (1992) extended this approach to include O_2 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 photorespiration and the rate relative to A_{CO_2} were lowered by 43% and by 57–70%, respectively, in 682 μmol mol^{-1} CO_2 compared with 344 μmol mol^{-1} CO_2.

Our results indicated that the suppression of photorespiration 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 photorespiration rates by CO_2 enrichment was accompanied by relatively small decreases in photorespiratory 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 photorespiratory cycle enzymes. It has been suggested

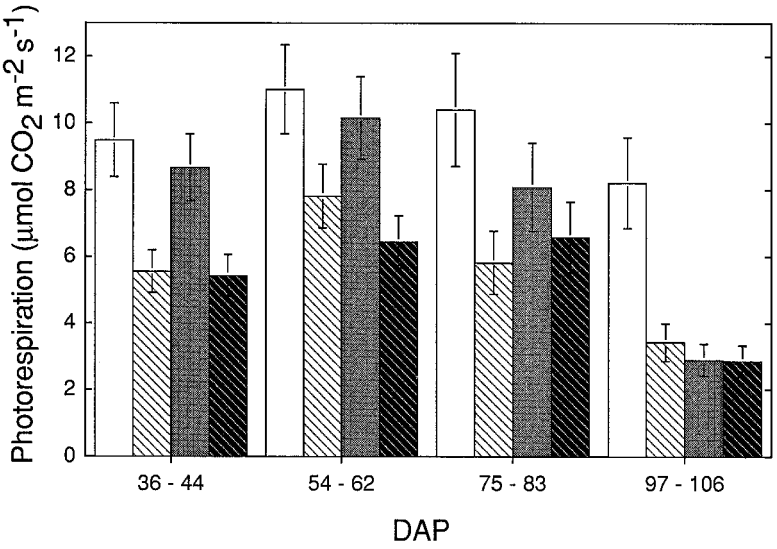

Fig. 2. E ects of elevated CO_2 and O_3 on photorespiration rates in the terminal mainstem leaf as determined by gas exchange measurements of plants in the laboratory during four sampling periods from 36–106 DAP. Photorespiration rates were calculated from measurements of A made at a range of CO_2 concentrations at 1300 μmol m^{-2} s^{-1} PAR and 28 °C. Treatments as in Fig. 1. Values are means $\pm$95% confidence intervals.

that, probably, there is no direct metabolic regulation of gene expression for photorespiratory cycle enzymes in response to CO_2, although indirect e ects of CO_2-enrichment on plant development could influence enzyme levels (Wallsgrove *et al.*, 1992). Previous short-term studies that used high levels of CO_2 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 Jolli e, 1980). Catalase activity in tobacco (*Nicotiana* sp.) leaves decreased 50% after plants were transferred from air to 1000 μmol mol^{-1} CO_2, but the activities of glycolate oxidase and hydroxypyruvate reductase were unchanged (Havir and McHale, 1989). No e ect was found on glycolate oxidase mRNA abundance in tomato leaves following exposure to 2000 μmol mol^{-1} CO_2 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} CO_2 compared with accumulation in 350 μmol mol^{-1} CO_2 (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} CO_2 than with ambient CO_2 (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 750 μmol mol^{-1} CO_2, and concurrent exposure to 80 nmol mol^{-1} O_3 exacerbated this e ect in the current year's needles (Polle *et al.*, 1993).

Lower levels of the photorespiration metabolite, serine, in the $+CO_2$ treatments suggested that photorespiration was decreased by CO_2 enrichment (Fig. 3d). However, it was unclear why only serine and not glycine levels were lower in the $+CO_2$ 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 e ects of elevated CO_2 on glycine-serine accumulation.

Effects of elevated O_3

The O_3 portion of the experiment indicated that A_{CO_2}, photorespiration rates, photorespiratory enzyme activities and serine levels eventually decreased in response to elevated O_3. At the 97–106 DAP sampling period, increased C_i levels in the O_3 treatments were insu cient 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 (Decleire *et al.*, 1984; Willekens *et al.*, 1994) or decreased (Decleire *et al.*, 1984) in leaves following exposure to O_3. Morré *et al.* (1990) reported that catalase activity in soluble extracts from O_3-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 CO_2 and O_3 (Polle *et al.*, 1993). In this study, photorespiratory enzyme activities and serine levels declined along with A_{CO_2} and calculated

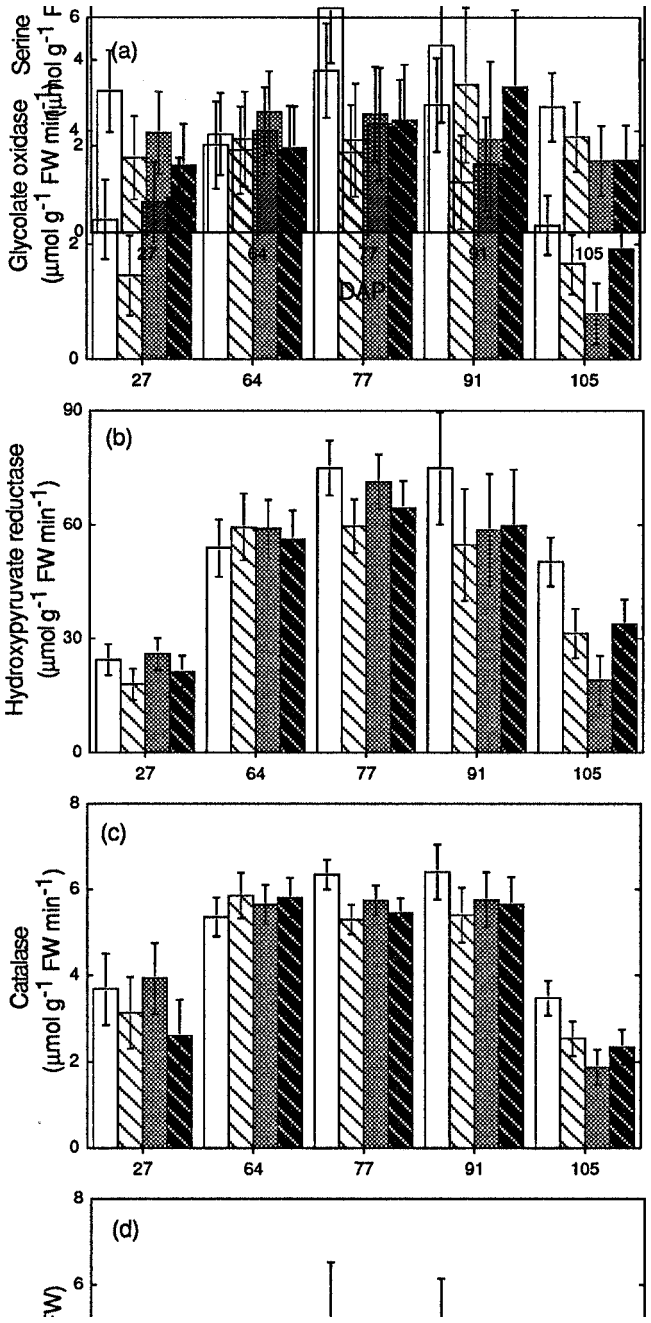

Fig. 3. E ects of elevated CO_2 and O_3 on the activity of glycolate oxidase (a), hydroxypyruvate reductase (b), catalase (c), and the level of serine (d) in mainstem leaves during five sampling periods from 27–105 DAP. Treatments as in Fig. 1. Values are means ±95% confidence intervals.

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.

Source	df	Glyox	Hydrd	Cat	AA	Gly	Ser
(A) Overall ANOVA results							
CO_2	1	0.06	0.07	0.01	0.86	0.68	0.02
O_3	1	0.88	0.28	0.04	0.55	0.52	0.03
$CO_2 \times O_3$	1	0.02	0.04	0.12	0.91	0.34	0.06
SP	4	0.001	0.001	0.001	0.001	0.02	0.01
$SP \times CO_2$	4	0.12	0.14	0.001	0.52	0.42	0.26
$SP \times O_3$	4	0.07	0.03	0.03	0.45	0.50	0.56
$SP \times CO_2 \times O_3$	4	0.18	0.01	0.02	0.71	0.68	0.11
(B) ANOVA results by sampling period							
27 DAP							
CO_2	1	0.15	0.02	0.03	0.04		
O_3	1	0.03	0.17	0.66	0.27		
$CO_2 \times O_3$	1	0.10[a]	0.56	0.26	0.52		
64 DAP							
CO_2	1	0.57	0.72	0.16	0.30		
O_3	1	0.70	0.80	0.58	0.90		
$CO_2 \times O_3$	1	0.74	0.27	0.46	0.38		
77 DAP							
CO_2	1	0.05	0.01	0.001	0.01		
O_3	1	0.67	0.86	0.18	0.08		
$CO_2 \times O_3$	1	0.14	0.22	0.04	0.01		
91 DAP							
CO_2	1	0.08	0.18	0.08	0.95		
O_3	1	0.32	0.42	0.51	0.11		
$CO_2 \times O_3$	1	0.11	0.14	0.15	0.18		
105 DAP							
CO_2	1	0.38	0.51	0.23	0.42		
O_3	1	0.02	0.001	0.001	0.04		
$CO_2 \times O_3$	1	0.003[b]	0.001[b]	0.003[b]	0.37		

[a]Pairwise comparison of CF-AA versus CF-CO_2 indicated significant di erence ($P = 0.05$).

[b]Pairwise comparison of CF-AA versus O_3-AA indicated significant di erences ($P < 0.001$); pairwise comparison of $CF + CO_2$ versus $O_3 + CO_2$ indicated no significant di erences ($P < 0.4$).

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 e ects of elevated O_3. Several previous studies have reported that elevated CO_2 alleviated the inhibitory e ects 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

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 CO_2 enrichment were important in counteracting O_3 toxicity. If so, these results indicated that elevated catalase levels were not required for this response.

Conclusions

Elevated CO_2 could promote productivity of C_3 plants partly through increased photoassimilation and suppressed photorespiration. In our experiment with soybean, CO_2 enrichment increased A and suppressed calculated photorespiration rates during both vegetative and reproductive growth. However, the moderate e ect of elevated CO_2 on photorespiratory 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 e ects of elevated O_3 on photorespiration generally coincided with O_3-induced declines in A_{CO_2}. Photorespiration-related parameters were not especially sensitive to elevated O_3. Carbon dioxide-enrichment generally diminished the detrimental e ects of elevated O_3 on A and photorespiration-related parameters.

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