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Effects of CO_2 Concentration on Rubisco Activity, Amount, and Photosynthesis in Soybean Leaves[1]

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ABSTRACT

Growth at an elevated CO_2 concentration resulted in an enhanced capacity for soybean (*Glycine max* L. Merr. cv Bragg) leaflet photosynthesis. Plants were grown from seed in outdoor controlled-environment chambers under natural solar irradiance. Photosynthetic rates, measured during the seed filling stage, were up to 150% greater with leaflets grown at 660 compared to 330 microliters of CO_2 per liter when measured across a range of intercellular CO_2 concentrations and irradiance. Soybean plants grown at elevated CO_2 concentrations had heavier pod weights per plant, 44% heavier with 660 compared to 330 microliters of CO_2 per liter grown plants, and also greater specific leaf weights. Ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) activity showed no response (mean activity of 96 micromoles of CO_2 per square meter per second expressed on a leaflet area basis) to short-term (~1 hour) exposures to a range of CO_2 concentrations (110–880 microliters per liter), nor was a response of activity (mean activity of 1.01 micromoles of CO_2 per minute per milligram of protein) to growth CO_2 concentration (160–990 microliters per liter) observed. The amount of rubisco protein was constant, as growth CO_2 concentration was varied, and averaged 55% of the total leaflet soluble protein. Although CO_2 is required for activation of rubisco, results indicated that within the range of CO_2 concentrations used (110–990 microliters per liter), rubisco activity in soybean leaflets, in the light, was not regulated by CO_2.

The concentration of CO_2 in the atmosphere is generally limiting to photosynthesis of C_3 plants (18). Increasing the CO_2 concentration to which plants are exposed provides more substrate for photosynthesis, as well as decreases the rate of photorespiration. Early experiments regarding the effects of CO_2 on plants were frequently short-term experiments, and often concerned mainly with yield (14). More recent experiments have included investigations of the effects of long-term exposure to various concentrations of CO_2 on the rate of photosynthesis (6, 18, 22, 28, 30). Results of experiments with CO_2 and photosynthesis vary with the species investigated (14). Nevertheless, by manipulating the CO_2 concentration, CO_2 can be used to probe the responses of various photosynthetic parameters and to aid in determining their role in regulation of photosynthesis.

The enzyme responsible for initiating C_3 photosynthesis, rubisco[3] has been the focus of much attention regarding the regulation of the rate of carbon entering the photosynthetic pathway. Rubisco catalyzes the carboxylation of RuBP, the first step in photosynthetic carbon assimilation. Extraction of rubisco from leaf tissue without further enzyme activation, and subsequent rapid assay of activity in the extract (often referred to as initial activity), is believed to provide a good estimate of the enzyme activation *in vivo* (20). Measurements of rubisco activity and RuBP levels have therefore been performed to study the regulation of the rate of leaf photosynthesis.

The objectives of the experiments reported here were to determine the response of rubisco activity to CO_2 in soybean leaflets following short-term and long-term exposure to various CO_2 concentrations, and to measure rubisco amount and kinetics, RuBP levels, and leaflet photosynthetic responses to CO_2 concentration. Plants were grown, from seed to maturity, under various CO_2 regimens in outdoor, controlled-environment growth chambers. By growing plants in canopies under natural solar irradiation and in reconstructed soil profiles, rather than under artificial light and in individual pots, plant responses should more closely resemble field crop responses, for the same CO_2 treatments.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Soybean plants (*Glycine max* L. Merr. cv Bragg) were grown from seed in six outdoor, sunlit, computer-managed, controlled-environment plant growth chambers at Gainesville, FL. The upper part of each chamber was constructed of clear acrylic and polyester film that transmitted 88% of the incoming solar irradiance. Chamber tops measured 2 m by 1 m in cross section by 1.5 m in height. The lower part of each chamber was a steel bin of the same cross section and 1 m in depth. It was filled with a reconstructed Arredondo fine sand soil profile from an adjacent site. The upper and lower portions of each chamber were separated by a gas tight seal, following seedling emergence, to prevent mixing of soil and aerial atmospheres. The CO_2 concentration was controlled from planting throughout the experiment to either 160, 220, 280, 330, 660, or 990 $\mu L\ L^{-1}$ in each chamber, dependent on the experiment. Carbon dioxide from high pressure cylinders was injected into the growth chambers to maintain treatment concentrations. Subatmospheric CO_2 concentrations were created by passing air through a system of large columns of Ascarite[4] to remove CO_2

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[3] Abbreviations: RuBP, ribulose 1,5-bisphosphate; rubisco, RuBP carboxylase/oxygenase; DAP, days after planting; C_i, intercellular CO_2 concentration; SLW, specific leaf weight.

[4] Mention of a trademark of a proprietary product in this paper does not imply endorsement by the University of Florida, the U.S. Department of Agriculture, or the U.S. Department of Energy.

to the experimental concentration. Removal of CO_2 was usually only necessary at low sunlight. At other times of the day sufficient amounts of CO_2 were removed by photosynthetic assimilation such that CO_2 was added back to the chambers, by computer-controlled injections, to the specific treatment concentrations. Measurements of CO_2 concentration were made with IR gas analyzers (12). The day/night dry bulb temperature was controlled to 31/23°C and the dew point temperature to 16°C in all experiments. Further chamber operating details may be found in Jones *et al.* (12, 13).

Results from experiments in 2 years are reported here. In the first year, plants were grown during the 1983 season at 330 and 660 μL CO_2 L^{-1}. Experimental measurements were made 8 weeks after planting. Data were collected, on plants from both CO_2 treatments, following approximately a 1 h exposure of the plants to CO_2 concentrations ranging from 110 to 880 μL L^{-1}. In the second year, during the 1984 season, the number of growth concentrations of CO_2 was expanded to include 160, 220, 280, 330, 660, and 990 μL L^{-1}. No exposures to CO_2 concentrations other than the growth concentration were conducted in these experiments. Experimental measurements were made 5 weeks after planting.

Leaflet tissue for rubisco assays and RuBP determinations was collected under high irradiance at mid-day by removing 20 to 25 fully expanded individual leaflets from the upper nonshaded portion of the canopy. Leaflets were rapidly (≤2 s) plunged into liquid N_2. Frozen leaf tissue was ground in a liquid N_2 cooled mortar and the powder stored at liquid N_2 temperature until subsequent laboratory analysis.

Leaflet Photosynthesis. Leaflet photosynthetic rates (net carbon exchange rates) per unit leaflet area of plants grown at 330 and 660 μL CO_2 L^{-1} were measured at their respective growth CO_2 concentrations as solar irradiance (400–700 nm) varied from 70 to 1050 μmol quanta $m^{-2}s^{-1}$ (measured at the leaflet level). Leaflet photosynthetic rates were also measured at high solar irradiance as the CO_2 concentration was varied over a range from 110 to 880 μL L^{-1}. In this experiment, photosynthetic rate data were collected between 1100 and 1430 Eastern Standard Time, when solar irradiance at the leaflet level was at least 1000 μmol quanta $m^{-2}s^{-1}$.

Photosynthetic rates were measured using two individual leaflet chambers located within each of the 330 and 660 μL CO_2 L^{-1} chambers. Leaflet chambers were constructed of the same acrylic and polyester film as the plant growth chambers, with an internal volume of 0.375 L. Air circulating through the leaflet chambers originated in the respective growth chambers. Chilled water circulating through the acrylic frame kept leaflet chamber air at the desired temperature. Measurements of photosynthetic rate response to CO_2 were made on leaflets 56 to 60 DAP, during the 1983 season, when plants were at the beginning of the seed filling stage of development. Response of photosynthesis to irradiance was measured 76 DAP. Leaflet intercellular CO_2 concentration (C_i) was calculated from leaflet photosynthetic rate, conductance to CO_2 diffusion, and ambient CO_2 concentration, using the method of Farquhar and Sharkey (8).

Rubisco Assay. Rubisco activity as reported here refers to the carboxylase activity of the enzyme. Except for determination of kinetics, the reported rubisco activity was determined in rapidly extracted and assayed leaflet crude extracts (initial activity), and not $MgCl_2$/$NaHCO_3$ incubated extracts (total activity). For assay of rubisco activity, a quantity of liquid N_2 frozen leaflet powder (equivalent to 100–170 mg dry weight) was homogenized in 10 mL of 50 mM Tris-HCl (pH 8.5), 5 mM DTT, 0.1 mM EDTA, and 1.5% (w/v) PVP-40 for 60 s at 0°C. An aliquot was reserved on ice for Chl and soluble protein determinations, and the remainder centrifuged at 12,000g for 3 min at 4°C. A 50 μL aliquot of the supernatant was assayed inmmediately. Assay buffer consisted of 50 mM Tris-HCl (pH 8.5), 5 mM DTT, 0.1 mM EDTA, 10 mM $MgCl_2$ 0.5 mM RuBP, and 20 mM $NaH^{14}CO_3$ (0.5 μCi μmol^{-1}). The assay vials were sealed with septum caps and flushed with N_2 for 10 min prior to adding $NaH^{14}CO_3$. Reactions were initiated by addition of the centrifuged crude extract and terminated after 45 s by addition of 6 N HCl. Assays were performed in a total volume of 1 mL at 30°C. An aliquot of the acidified assay mixture was dried and acid-stable ^{14}C products were determined by liquid scintillation spectrometry. For determination of apparent K_m (CO_2) and V_{max}, rubisco in the crude extract was preactivated with $MgCl_2$ and $NaH^{14}CO_3$. A quantity of frozen leaflet powder (equivalent to 70–150 mg dry weight) was homogenized in 5 mL of 100 mM Tris-HCl (pH 8.0), 5 mM DTT, 10 mM isoascorbate, 5 mM $MgCl_2$ and 1.5% (w/v) PVP-40. Centrifugation was as described above. An aliquot of the supernatant was activated by incubation in 50 mM Tris-HCl (H 8.0), 5 mM DTT, 10 mM isoascorbate, 5 mM $MgCl_2$ and 10 mM $NaH^{14}CO_3$ (0.2 μCi μmol^{-1}) for 45 min at 0°C. Assay buffer was prepared CO_2-free and consisted of 50 mM Tris-HCl, 5 mM DTT, 5 mM $MgCl_2$, and 10 mM isoascorbate. Buffer was purged with N_2 for 15 min at pH 3.1. The pH was then raised to 8.0 with CO_2-free NaOH. To complete the assay buffer, 0.5 mM RuBP, carbonic anhydrase (54 Wilbur-Anderson units), and 0.25 to 10 mM $NaH^{14}CO_3$ (0.2 μCi μmol^{-1}) were added. Buffer was flushed with N_2 for an additional 10 min prior to adding $NaH^{14}CO_3$. The quantity of $NaH^{14}CO_3$ carried over into the assay with the addition of activated crude extract was included in calculating the final concentration of $NaHCO_3$. Assays were performed in a total volume of 1 mL at 30°C and were terminated after 45 s by addition of 6 N formic acid in methanol. Determination of acid-stable ^{14}C products was as described above. In calculating CO_2 concentration from the $NaHCO_3$ concentration, a pK for carbonic acid of 6.095 was used. During the assays, bicarbonate consumption was always less than 20% and usually less than 10%. The apparent K_m and V_{max} values were calculated from Lineweaver-Burke plots.

The amount of rubisco protein was determined based on the method of Servaites *et al.* (24). This method quantifies Coomassie brilliant blue R stained protein, following SDS-PAGE, by spectrophotometric measurement of the eluted dye. A quantity of liquid N_2 frozen leaflet powder (equivalent to 75 mg dry weight) was homogenized in extract buffer consisting of 50 mM Tris-HCl (pH 8.0), 5 mM DTT, and 5 mM $MgCl_2$. Following centrifugation at 12,000 *g* for 3 min, 0.2 mL supernatant was added to 0.2 mL sample buffer (500 mM Tris-HCl [pH 6.8], 10% [w/v] SDS, 100 mM DTT, 0.1% [w/v] bromophenol blue, and 20% [v/v] glycerol) and placed in boiling water for 5 min. Aliquots equivalent to approximately 10 μg protein were subjected to 12% (w/v) SDS-PAGE. Gels were then stained with Coomassie brilliant blue R and destained in 7% (v/v) acetic acid and 10% (v/v) methanol. Stained bands in the gel representing both the large and small rubisco subunits were excised and the dye eluted in 1% (w/v) SDS. Following centrifugation at 2,000*g* for 3 min, absorbance was read at 600 nm. Purified tobacco rubisco was included with each measurement as a standard to calibrate the procedure.

RuBP Determination. Levels of RuBP were measured enzymatically in leaflet tissue previously frozen in liquid N_2 using the method of Vu *et al.* (29). Assays were performed at 30°C and terminated after 1 h.

Chl, Soluble Protein, Specific Leaf Weight, and Pod Weight Determinations. Chl was extracted in 80% (v/v) acetone and determined by the method of Arnon (1). Soluble protein determinations were made by a dye-binding method (Bio-Rad, Richmond, CA). Specific leaf weight was determined by drying fully expanded leaflets of known surface area, collected from the upper nonshaded portion of the canopy, to constant weight at 70°C.

Average pod dry weight per plant was measured three times during the season. At 49, 91, and 108 DAP plants (12, 4, and 56 plants, respectively) were harvested from each chamber and all viable pods were removed. Pods were dried to constant weight at 70°C.

Analysis of Statistical Significance. To determine the statistical significance of experimental results, simple linear regressions were performed. Comparison of slopes and intercepts between treatments and comparison of slopes to zero were used as tests of significance. A quadratic regression was performed on the RuBP data. All tests of significance were made at the 5% level.

RESULTS

Growth of soybean plants at an elevated CO_2 concentration resulted in greater leaflet photosynthetic rates, on a leaflet area basis, compared to plants grown at atmospheric CO_2 concentration. Photosynthetic rates increased with increasing solar irradiance in plants grown and measured at both 330 and 660 μL CO_2 L^{-1} (Fig. 1). Leaflets grown and measured at high CO_2 had greater photosynthesis rates at all irradiance levels, suggesting a higher quantum yield.

When measured at high irradiance levels ($\geq$1000 μmol quanta m^{-2} s^{-1}), plants grown at 660 μL CO_2 L^{-1} had greater leaflet photosynthetic rates than plants grown at 330 μL CO_2 L^{-1}, across a wide range of C_i values (Fig. 2). Photosynthetic rates of the high CO_2 grown leaflets were as much as 150% greater (at a C_i of about 420 μl CO_2 L^{-1}) than the 330 μl CO_2 L^{-1} grown leaflets. Photosynthetic response to CO_2 was measured during the seed filling stage, following 8 weeks of growth at either 330 or 660 μL CO_2 L^{-1}. High CO_2 grown leaflets had greater photosynthetic rate responses to increasing CO_2 and also greater maximum rates under these experimental conditions. Photosynthetic rates of leaflets grown at 330 μL CO_2 L^{-1} saturated at a C_i value of approximately 500 μL L^{-1}. Rates of high CO_2 grown leaflets did not C_i saturate within the range of CO_2 concentrations used. Arrows in Figure 2 indicate rates (as a function of C_i) measured at the respective growth CO_2 concentrations. Leaflets grown and measured at 660 μL CO_2 L^{-1} had greater photosynthesis rates (39.5 μmol CO_2 m^{-2} s^{-1}) than those grown and measured at 330 μL CO_2 L^{-1} (16.9 μmol CO_2 m^{-2} s^{-1}). Extrapolation gave estimated CO_2 compensation points, as C_i of approximately 21 and 29 μL L^{-1}, respectively, from the 330 and 660 μL CO_2 L^{-1} grown leaflets. Extrapolated compensation points will, however, depend on how the curves are drawn.

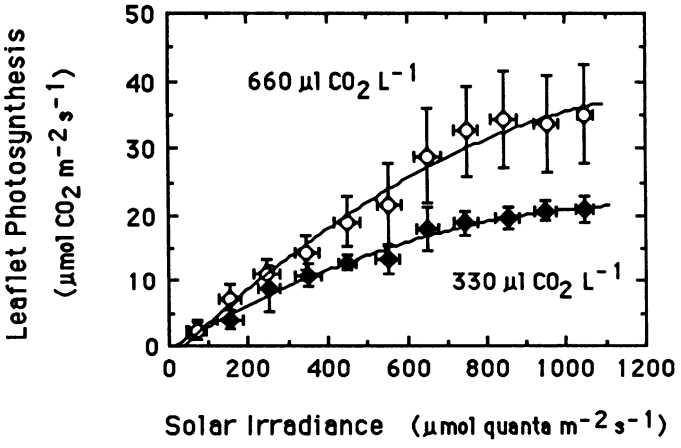

FIG. 1. Leaflet photosynthetic rates (leaflet area basis) *versus* solar irradiance for soybean plants grown and measured at 330 and 660 μL CO_2 L^{-1}. Each data point represents the mean of 4 to 32 total observations of two leaflets at each CO_2 concentration. The SD of the data is shown as horizontal and vertical bars through each symbol. Solar irradiance values are at the leaflet level. Measurements were made 76 DAP during the 1983 season.

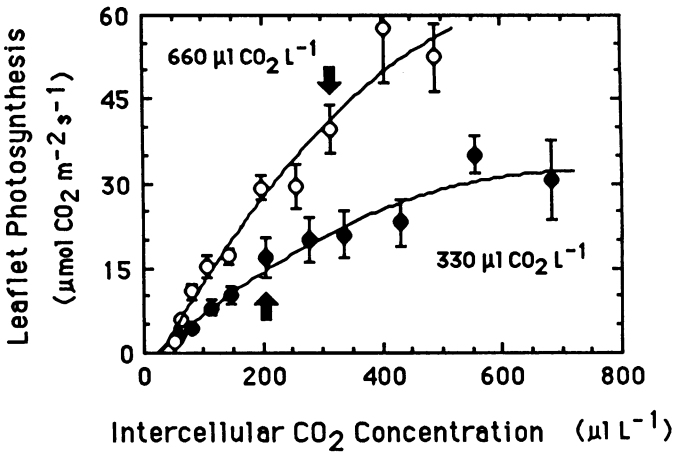

FIG. 2. Leaflet photosynthetic rates (leaflet area basis) versus intercellular CO_2 concentration for soybean plants grown at 330 and 660 μL CO_2 L^{-1}. Rates were recorded when solar irradiance at the leaflet level was at least 1000 μmol quanta m^{-2} s^{-1}. Each data point represents the mean of 4 to 20 total observations of two leaflets at each growth CO_2 concentration. The SD of the data is shown as vertical bars through each symbol. Arrows indicate the rates measured at the respective growth CO_2 concentration. Measurements were made 56 to 60 DAP during the 1983 season.

Table I. *Rubisco Activity in Crude Extracts of Leaflets Exposed to Concentrations of CO_2 from 110 to 880 μL L^{-1} for ~1 h*

Plants were grown at 330 or 660 μL CO_2L^{-1}. Leaflet tissue was collected for assay immediately following leaflet photosynthesis measurements (Fig. 2). Activity is expressed on a leaflet area basis. Data were collected during the 1983 season. Mean ± SD is shown.

External CO_2 Concentration	Rubisco Activity at Growth CO_2 Concentration:	
	330 μL L^{-1}	660 μL L^{-1}
μL L^{-1}	μmol CO_2 m^{-1} s^{-1}	
110	92 ± 2	94 ± 4
220	106 ± 3	90 ± 3
330	106 ± 4	122 ± 1
550	87 ± 1	88 ± 3
660	93 ± 1	104 ± 3
880	94 ± 1	90 ± 3

Leaflet tissue was collected immediately following photosynthesis measurements (Fig. 2) for assay of rubisco activity. Activity, expressed on a leaflet area basis, was found to be independent of CO_2 concentration. As shown in Table 1, rubisco activity was not affected by short-term exposures (~1 h) to external CO_2 concentrations ranging from 110 to 880, μL L^{-1}, in plants grown in 330 (mean activity of 96 μmol CO_2 m^{-2} s^{-1}) or 660 μL CO_2 L^{-1} (mean activity of 98, μmol CO_2 m^{-2} s^{-1}). In addition, rubisco activity was very similar at both growth CO_2 concentrations, for any given external CO_2 concentration, indicating no significant effect of growth concentration.

In the second year of experiments, in which soybean plants were grown at CO_2 concentrations from 160 to 990 μL L^{-1}, similar results regarding growth concentration of CO_2 were observed. In Figure 3, rubisco activity is expressed on a leaflet soluble protein basis and is plotted against growth CO_2 concentration. Enzyme activity was found to be independent of growth CO_2 concentration, and ranged between 0.91 and 1.10 μmol CO_2 min^{-1} mg^{-1} protein (mean activity of 1.01 μmol CO_2 min^{-1} mg^{-1} protein). Rubisco activation state did not decrease at a CO_2

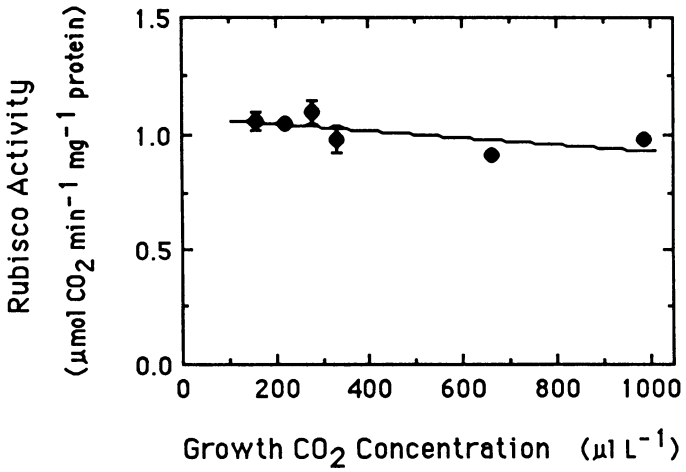

FIG. 3. Rubisco activity in crude extracts of leaflets grown at 160, 220, 280, 330, 660, and 990 μL CO_2 L^{-1}. Activity is expressed on a leaflet soluble protein basis. The SD of the data is shown as bars through each symbol. Where no bar is visible, the SD is smaller than the symbol. Leaflets were harvested for analysis 34 DAP during the 1984 season.

concentration equal to one-half of the atmospheric concentration, nor did it change at a CO_2 concentration three times that of atmospheric. Total rubisco activity (data not shown), measured following a 5 min incubation with 10 mM $MgCl_2/NaHCO_3$ at 30°C, was also not affected by short-term (Table I experiment) or long-term (Fig. 3 experiment) exposures to any of the CO_2 concentrations in these experiments.

Total leaflet soluble protein did not change with growth CO_2 concentration. On a leaflet area basis, protein levels were unchanged (Table II). The percent of total leaflet soluble protein represented by rubisco protein was also unaffected by increasing levels of CO_2 during growth from 160 to 990 μL L^{-1} (Table II). Rubisco protein averaged 55% of the soluble protein in the leaflet for all growth CO_2 concentrations. Leaflet soluble protein was statistically independent of CO_2. The kinetic parameters apparent K_m (CO_2) and V_{max}, as a function of growth CO_2 are also shown in Table II. As growth CO_2 was increased from 160 to 990 μL L^{-1}, the apparent K_m (CO_2) decreased from 9.7 to 8.4 μM. This represents a small, but statistically significant, 13% decrease with a six-fold increase in CO_2 concentration. Doubling the CO_2 from 330 to 660 μL L^{-1} resulted in only a 6% change in apparent K_m (CO_2). The V_{max} values (expressed on a leaflet soluble protein basis) were statistically independent of CO_2 concentration (Table II), although there was a 19% difference between the highest and lowest values.

There was a significant linear increase in SLW with increasing CO_2 (Table III). Leaflets were 50% heavier following growth at 990 compared to 160 μL CO_2 L^{-1}. In contrast leaflet soluble protein, expressed in a dry weight basis, decreased significantly with increasing CO_2 (Table III) and was 39% lower at the highest CO_2 concentration compared to the lowest CO_2 concentration. This decrease in soluble protein, on a dry weight basis but not on a leaflet area basis (Table II), is due to the response of SLW to CO_2. Data in Tables II and III indicate that, whereas soluble protein on an area basis did not change with CO_2, it decreased with increasing CO_2 when expressed on a dry weight basis.

When rubisco activity is expressed on a dry weight basis, activity decreases with increasing growth CO_2 concentration (Fig. 4). This response to CO_2 is different from the response of activity when expressed on a soluble protein basis (Fig. 3) or leaflet area basis (Table I). In both of the latter cases, rubisco activity was independent of CO_2 concentration. In Figure 4, activity decreased 41% as growth CO_2 was increased from 160 to 990 μL L^{-1}. The response of rubisco activity to CO_2 (Fig. 4) is similar to the response of leaflet soluble protein to CO_2 (Table III) when both are expressed on a leaflet dry weight basis.

As growth concentration of CO_2 was increased from 160 to 990 μL L^{-1}, steady-state RuBP levels decreased from 207 to 73 nmol mg^{-1} Chl (Fig. 5). There were no statistically significant differences in Chl levels with growth CO_2 concentration, when expressed on a leaflet area basis. The decrease in RuBP, with increasing CO_2, was greatest at CO_2 concentrations below 330 μL L^{-1}. Above 660 μL CO_2 L^{-1} there was no further response to CO_2. The concentration of RuBP (Fig. 5) was calculated assuming a chloroplast stromal volume of 25 μL mg^{-1} Chl·RuBP declined from a high of 8.2 mM, at 160 μL CO_2 L^{-1}, to 2.9 mM at 990 μL CO_2 L^{-1}.

Levels of RuBP were also measured (data not shown) in the same tissue samples in which rubisco activity was assayed following growth at either 330 or 660 μL CO_2 L^{-1} and exposed (~1 h) to a range of external CO_2 concentrations (Table I experiment). The same response of RuBP levels were observed as is shown in Figure 5. That is, RuBP decreased with increasing external CO_2 concentrations in plants grown at both 330 and 660 μL CO_2 L^{-1}.

Pod dry weight per plant was measured in plants grown at 330 and 660 μL CO_2 L^{-1} three times during the season (Table IV). The average pod weight increased as the season progressed at both CO_2 concentrations. Pod weight was always heavier with high CO_2 grown plants. Plants grown at 660 μL CO_2 L^{-1} averaged 44% heavier pod dry weight per plant than plants grown at 330 μL CO_2 L^{-1}.

DISCUSSION

When measured at the respective growth CO_2 concentrations, soybean leaflets grown at elevated CO_2 had greater photosyn-

Table II. *Leaflet Soluble Protein, Rubisco Protein, and Apparent $K_m(CO_2)$ and V_{max} of Rubisco from Crude Extracts of Leaflets Grown at Six CO_2 Concentrations*

Percent rubisco protein and V_{max} are expressed on a leaflet total soluble protein basis. Rubisco protein was quantified by elution of dye from stained large and small subunits separated by SDS-PAGE. Kinetic parameters were determined from assays of preactivated ($MgCl_2/NaHCO_3$) rubisco. All data were collected from leaflets harvested 34 DAP during the 1984 season. Mean ± SD is shown.

Growth CO_2 Concentration	Leaf Soluble Protein	Rubisco Protein	Apparent $K_m(CO_2)$	V_{max}
$\mu L\ L^{-1}$	gm^{-2}	%	μM	$\mu mol\ CO_2\ min^{-1}\ mg^{-1}$ protein
160	2.53 ± 0.01	56	9.7 ± 0.3	1.38 ± 0.01
220	3.23 ± 0.02	54		
280	2.58 ± 0.02		9.7 ± 0.7	1.60 ± 0.03
330	2.31 ± 0.01	57	9.4 ± 0.7	1.51 ± 0.03
660	2.28 ± 0.04	54	8.8 ± 0.7	1.29 ± 0.03
990	2.29 ± 0.01	55	8.4 ± 0.5	1.54 ± 0.02

Table III. *Specific Leaf Weight and Leaflet Soluble Protein (Dry Weight Basis) of Soybean Plants Grown at Six CO_2 Concentrations*

All data were collected from leaflets harvested 34 DAP during the 1984 season. Mean ± SD is shown.

Growth CO_2 Concentration	Specific Leaf Weight	Leaf Soluble Protein
$\mu L\ L^{-1}$	$g\ dry\ wt\ m^{-2}$	$mg\ g^{-1}\ dry\ wt$
160	20.3 ± 2.1	124 ± 1
220	20.9 ± 1.7	154 ± 2
280	21.4 ± 2.4	120 ± 1
330	21.4 ± 2.4	108 ± 1
660	26.6 ± 5.2	85 ± 2
990	30.5 ± 5.2	75 ± 1

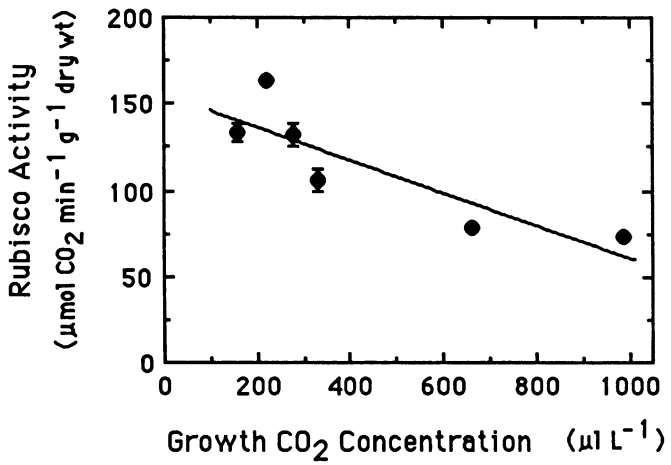

FIG. 4. Rubisco activity in crude extracts of leaflets grown at 160, 220, 280, 330, 660, and 990 $\mu L\ CO_2\ L^{-1}$. Activity is expressed on a leaflet dry weight basis. The SD of the data is shown as bars through each symbol. Where no bar is visible, the SD is smaller than the symbol. Leaflets were harvested for analysis 34 DAP during the 1984 season.

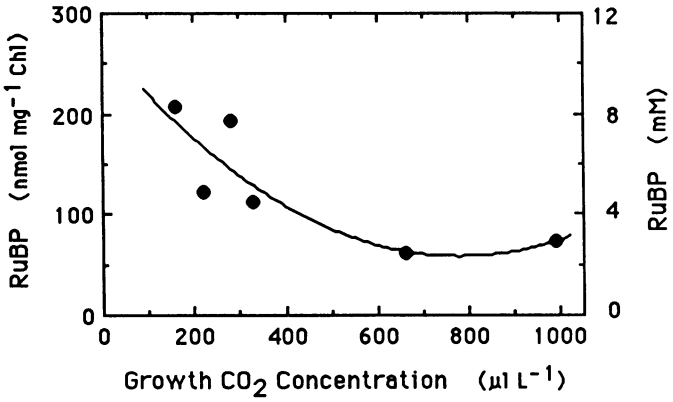

FIG. 5. Steady-state RuBP levels in leaflets grown at 160, 220, 280, 330, 660, and 990 $\mu L\ CO_2\ L^{-1}$. Concentration (mM) of RuBP was calculated assuming a stromal volume of 25 $\mu L\ mg^{-1}$ Chl. Bars representing the SD of the data cannot be seen as they are smaller than the symbols. Leaflets were harvested for analysis, under conditions of high solar irradiance (1100 $\mu mol\ quanta\ m^{-2}\ s^{-1}$), 34 DAP during the 1984 season.

thetic rates, across the full range of daily solar irradiance, than those grown at atmospheric CO_2 concentrations. When measured at saturating irradiance and varying intercellular CO_2 concentrations, elevated CO_2 grown leaflets always had greater photosynthetic rates. In all experiments reported here where leaflet photosynthesis was measured, plants grown at elevated CO_2 had rates greater than plants grown at atmospheric concentrations of CO_2. Growth of soybean plants at elevated CO_2 resulted, under our conditions during the seed filling stage, in an enhanced capacity for leaflet photosynthesis. In contrast, *Phaseolus vulgaris* (28) and *Nerium oleander* (6) plants grown at atmospheric CO_2 concentrations had greater leaf photosynthetic rates, across a range of C_i values, than did elevated CO_2 grown plants. Wong (30), working with *Gossypium hirsutum*, reported that plants grown at atmospheric CO_2 concentrations had greater leaf photosynthesis rates, when measured at C_i values below approximately 450 $\mu L\ L^{-1}$, than plants grown at 640 $\mu L\ CO_2\ L^{-1}$, although above this C_i value the elevated CO_2 grown leaves had the greater photosynthetic rates. Radin *et al.* (22) recently reported a decrease in unstressed *G. hirsutum* leaf photosynthetic rate responses to C_i with high CO_2 grown plants, relative to ambient CO_2 grown plants, as the season progressed. It thus appears that leaf photosynthetic response to C_i is influenced not only by the CO_2 concentration during growth, but also the stage of plant development, plant species, and possibly other variables.

Table IV. *Average Pod Dry Weight per Plant for Soybean Plants Grown at 330 and 660 $\mu L\ CO_2 L^{-1}$*

Measurements were made three times during the 1983 season. Plants had reached physiological maturity by 108 DAP.

Days after Planting	Pod Weight		
	330 $\mu L\ CO_2 L^{-1}$	660 $\mu L\ CO_2 L^{-1}$	660/330
	$g\ dry\ wt\ plant^{-1}$		
49	0.08	0.12	1.50
91	7.52	10.42	1.38
108	11.28	16.33	1.44

Assays of rubisco indicated the activation state *in vivo* (initial activity) did not respond to changes in CO_2 concentration. No difference in activity, on a leaflet area or soluble protein basis, was observed following short-term (~1 h) exposure to a wide range of CO_2 concentrations, in 8 week old plants. Nor was there a difference in activity, on a leaflet area or soluble protein basis, among plants grown at CO_2 concentrations from 160 to 990 $\mu L\ L^{-1}$, when measured in five or 8 week old plants. At high irradiance levels, short-term increases in CO_2 from 110 to 880 $\mu L\ L^{-1}$ caused a marked increase in leaflet photosynthetic rate but no change was observed in rubisco activity. The lack of response of rubisco activation state, in the light, to short-term changes in CO_2 concentration has also been observed in *Arabidopsis* (23) and *Triticum aestivum* seedlings (19) grown in chambers under artificial light. Rubisco activity was also unchanged as CO_2 concentration during growth was increased (Table I) (6). There are, however, other reports of activity decreasing with increasing growth concentration of CO_2 in *P. vulgaris* (28) and in *G. hirsutum* (30), when activity was expressed on a leaf area basis. Comparison of results presented here with previous reports in the literature suggests that the enhancement of soybean leaflet photosynthetic efficiency following growth at elevated CO_2 does not appear to be ubiquitous.

The observation that at high irradiance, changes in CO_2 (from 110 to 880 $\mu L\ L^{-1}$) did not cause a response in rubisco activation is consistent with the low K_{act} (CO_2) of rubisco reported to occur *in vivo* (21). Soybean rubisco activation *in vivo* is effectively CO_2-saturated at a concentration less than 110 $\mu L\ L^{-1}$, measured external to the leaf. von Caemmerer and Edmondson (27) found *Raphanus sativus* rubisco to be 50% activated at a C_i of approximately 10 $\mu L\ L^{-1}$. In the experiments reported here, the only response of rubisco activity to CO_2 was observed when activity was expressed on a leaflet dry weight basis. This resulted from an increase in SLW with increasing CO_2.

Leaflet soluble protein, on an area basis, and the percent of leaflet soluble protein comprising rubisco, remained unchanged as the growth concentration of CO_2 was either decreased below or increased above atmospheric concentrations. In all CO_2 treatments, just over one-half of the soluble protein in the leaflet was rubisco. The apparent K_m (CO_2) decreased slightly as CO_2 was increased. Yeoh *et al.* (31) similarly reported a minimal effect of CO_2 concentration on K_m (CO_2) of *G. hirsutum* rubisco. V_{max} values were not dependent on the growth CO_2 concentration. Data presented here indicate that within the range of CO_2 concentrations used, rubisco activity in soybean leaflets in the light is not regulated by CO_2. However, as expected increasing the CO_2 concentration did result in an increase in leaflet photosynthetic rates. Carbon dioxide is a substrate for photosynthesis, and high CO_2 therefore provides more substrate and also reduces the rate of photorespiration.

Whereas neither rubisco protein nor activity changed with varying CO_2 concentration, steady-state levels of RuBP at high irradiance did respond to changes in growth CO_2 concentration. A decrease in RuBP with increasing CO_2 occurred at concentrations below 330 μL CO_2 L^{-1}, but at concentrations higher than 660 μL CO_2 L^{-1} there was no change in RuBP. As in the present experiment, Vu *et al.* (29) failed to find a difference in leaflet RuBP among soybean plants grown at higher than atmospheric CO_2 concentrations (450 and 800 μL CO_2 L^{-1}). Decreases in RuBP levels have been reported for *P. vulgaris* (2) and *Xanthium stumarium* (16) with plants grown at atmospheric CO_2 concentrations and exposed to various external CO_2 concentrations.

The higher rates of leaflet photosynthesis reported here for plants grown at elevated CO_2 (Fig. 2) do not appear to be due to differences in rubisco amount or activity, nor to differences in RuBP levels. Though the reduced RuBP levels may be the result of greater photosynthetic activity in leaflets at the higher CO_2 concentrations. It is puzzling that with the same calculated intercellular CO_2 concentration, higher rates of leaflet photosynthesis were observed in plants grown at elevated CO_2, compared to those grown at atmospheric concentrations of CO_2 yet the activity of rubisco (the enzyme that catalyzes the photosynthetic uptake of CO_2) remained unchanged.

Greater leaflet photosynthetic rates may be related to heavier pod weights per plant following growth at high CO_2. Enos *et al.* (7) reported greater leaf photosynthetic rates with soybean plants having heavier pod weights. Greater assimilate demand due to increased sink tissue, such as soybean pods, has been associated with increased photosynthesis in several species (9), although the mechanism(s) involved is (are) not well understood. During the seed filling stage, a greater pod weight commitment may require a higher photosynthetic rate. Another possible explanation for the difference in leaflet photosynthesis rates may be a difference in leaflet anatomy. There are data showing strong correlations between SLW and soybean leaflet photosynthesis (4, 5). Results from studies reported here show an increase in SLW with increasing CO_2 during growth. In soybean, growth at elevated CO_2 results in thicker leaflets with more palisade cells per unit leaflet area (11, 25). An increase in leaflet mesophyll cell number per unit leaflet surface area will result in an increase in the ratio of mesophyll cell surface area to unit leaflet surface area (A_{mes}/A), a response which has been reported to result in higher leaflet photosynthetic rates (17). Leadley *et al.* (15), however, failed to observe a change in A_{mes}/A between soybean plants grown at 348 and 645 μL CO_2 L^{-1}. If an increase in mesophyll cell surface area is responsible for greater leaflet photosynthesis rates in the high CO_2 grown plants (Fig. 2), then resistance to diffusion of intercellular CO_2 would appear to be substantial resistance. This has not been shown unequivocally to be the case.

It is of interest to compare the response of certain leaf characteristics to differences in the growth conditions of CO_2 and light. Short-term increases in CO_2 concentration or irradiance, over a relatively wide range, are typically accompanied by increases in the rate of leaf photosynthesis for C_3 plants. However, growth under various levels of CO_2 and irradiance can result in differential responses of soybean plant characteristics important in photosynthesis. Rubisco activity, on a leaflet area basis, was unchanged as CO_2 during growth was increased (Table I). But on the same basis, activity increased with increasing irradiance during growth (3, 4, 26). As CO_2 during growth was increased, leaflet soluble protein on a leaflet area basis was unchanged (Table II) (10). With increasing irradiance during growth, leaflet soluble protein increased (26). Increases in SLW have been observed with both increasing CO_2 (Table III) (10) and irradiance (4, 26) during growth. Thus, whereas increasing irradiance during growth results in more leaflet soluble protein and greater rubisco activity on a leaflet area basis, increasing CO_2 did not elicit similar responses. There appears to be a distinction between the response of a soybean leaflet to differences during growth in the CO_2 concentration, a parameter that is relatively constant in the field, and to differences in the intensity of light, a parameter that varies greatly on a daily basis.

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