Plant Physiol. (1987) 83, 573–578
0032-0889/87/83/0573/06/$01.00/0

Drought Stress and Elevated CO_2 Effects on Soybean Ribulose Bisphosphate Carboxylase Activity and Canopy Photosynthetic Rates[1]

Received for publication July 7, 1986 and in revised form October 9, 1986

J. Cu V. Vu[2], Leon H. Allen, Jr.*, and George Bowes
Department of Agronomy (J.C.V.V., L.H.A., Jr.), *United States Department of Agriculture, Agricultural Research Service* (L.H.A., Jr.), *and Department of Botany* (G.B.), *University of Florida, Gainesville, Florida 32611*

ABSTRACT

Soybean (*Glycine max* [L.] cv Bragg) was grown at 330 or 660 microliters CO_2 per liter in outdoor, controlled-environment chambers. When the plants were 50 days old, drought stress was imposed by gradually reducing irrigation each evening so that plants wilted earlier each succeeding day. On the ninth day, as the pots ran out of water CO_2 exchange rate (CER) decreased rapidly to near zero for the remainder of the day. Both CO_2-enrichment and drought stress reduced the total (HCO_3^-/Mg^{2+}-activated) extractable ribulose-1,5-bisphosphate carboxylase (RuBPCase) activity, as expressed on a chlorophyll basis. In addition, drought stress when canopy CER values and leaf water potentials were lowest, reduced the initial (nonactivated) RuBPCase activity by 50% compared to the corresponding unstressed treatments. This suggests that moderate to severe drought stress reduces the *in vivo* activation state of RuBPCase, as well as lowers the total activity. It is hypothesized that stromal acidification under drought stress causes the lowered initial RuBPCase activities. The $K_m(CO_2)$ values of activated RuBPCase from stressed and unstressed plants were similar; 15.0 and 12.6 micromolar, respectively. RuBP levels were 10 to 30% lower in drought stressed as compared to unstressed treatments. However, RuBP levels increased from near zero at night to around 150 to 200 nanomoles per milligram chlorophyll during the day, even as water potentials and canopy CERs decreased. This suggests that the rapid decline in canopy CER cannot be attributed to drought stress induced limitations in the RuBP regeneration capability. Thus, in soybean leaves, a nonstomatal limitation of leaf photosynthesis under drought stress conditions appears due, in part, to a reduction of the *in vivo* activity of RuBPCase. Because initial RuBPCase activities were not reduced as much as canopy CER values, this enzymic effect does not explain entirely the response of soybean photosynthesis to drought stress.

The global atmospheric CO_2 concentration, presently at about 345 μl/L, is increasing at an annual rate greater than 1 μl/L and could double by the end of the next century (2). Climate modelers have predicted that a doubling of CO_2 concentration could lead to changes in global climate, including precipitation patterns, and therefore could result in increased drought conditions in some areas of the world (17).

Both short- and long-term effects of CO_2 enrichment on CER,[3] overall growth, and reproductive capability of a variety of plant species have been documented (12, 21, 23, 25). For a number of crops, increased CO_2 concentration during growth enhances CER and final yield. An increase in drought stress, in contrast, is followed ultimately by decreasing CER, but the mechanisms contributing to this reduction are still not well understood. Part of the reduction in CER has been attributed to stomatal closure which occurs as drought stress develops (8). Nonstomatal components involved in this CER reduction, when leaf water potentials were lowered, also have been reported. These nonstomatal factors include decreases in activities of PSI and PSII (9, 14, 18) and increases in photorespiration and dark respiration as a proportion of net photosynthesis (15). Studies with leaf discs, isolated protoplasts, and intact chloroplasts using sorbitol, mannitol, or PEG to induce osmotic stress have indicated effects on assimilation products, photosynthetic enzyme activities, and stromal pH (3–5, 13, 24). Since the carboxylation step of photosynthesis is one of the major biochemical processes of carbon assimilation and contributes substantially to mesophyll resistance, any effects of drought stress on this nonstomatal component could be of crucial importance.

In this study, soybean was grown under natural solar irradiance with CO_2 levels of 330 or 660 μl/L and then gradually subjected to increasing drought stress. The results revealed that a reduction in canopy CER due to drought stress was associated with reduced extractable RuBPCase activity and a substantial reduction in RuBPCase activation for both CO_2 treatments. There was no evidence that RuBP regeneration was limiting under drought stress. Furthermore, growth under CO_2 enrichment did not alleviate the detrimental effects of moderate to severe drought stress on canopy CER.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Soybeans (*Glycine max* [L.] Merr. cv Bragg) were planted on March 29, 1983 at Gainesville, FL in acrylic cylindrical pots (10 cm i.d. and 58 cm high) containing a rooting medium of equal mixture (v/v) of fine

[1] Supported in part by the United States Department of Energy, Carbon Dioxide Research Division Interagency Agreement No. DE-AI01-81ER60001 with the United States Department of Agriculture, Agricultural Research Service. Florida Agricultural Experiment Station Journal Series No. 7407.

[2] Present address: Horticultural Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, 2120 Camden Road, Orlando, FL 32803.

[3] Abbreviations: CER, CO_2 exchange rate; EST, eastern standard time; LAI, Leaf area index; RuBP, ribulose-1,5-bisphosphate; RuBPCase, ribulose-1,5-bisphosphate carboxylase.

sand and vermiculite with additional major and minor nutrient elements. Complete nutrients were applied again on May 9 via irrigation. Four seeds were planted in each pot. The pots were placed immediately in four acrylic 2-m × 1-m (ground area) × 1.5 m (height) controlled-environment growth chambers located outdoors as described previously (12). Day/night air temperature was 27/23°C and dewpoint temperature was maintained at 12°C both day and night. The CO_2 concentration was controlled to either 330 (two chambers) or 660 (two chambers) μl/L. Eighteen pots were placed in each chamber. The plants were thinned to 2 plants per pot on April 6 and to 1 plant per pot on April 12. A total of 10 pots were removed and whole plants were sampled on May 11 and 8 plants on May 17 for other studies, so that 14 pots remained in one each of the 330 μl/L and the 660 μl/L chambers and 13 pots remained in the other two chambers.

On May 18, 1983, 50 d after seeding, drought stress was imposed in one each of the 330 and 660 μl/L CO_2 treatments (with 14 remaining pots) by replacing each night only about 85% of the previous daytime water loss by transpiration. Water loss was determined each evening (1600–1700 EST) by weighing each pot with an electronic balance and subtracting each weight from the pot weight plus water added of the previous evening. The average transpirational water loss per pot was computed and the 85% replacement calculated and added to the drought stress pots. Thus, drought stress developed within a few days and became progressively more severe as wilting occurred earlier during each succeeding day. The unstressed pots were watered fully each evening during this period. Two more plants per chamber were removed for sampling on each of May 21 and May 25, so that 10 plants per chamber remained in the drought stress treatment and 9 plants per chamber remained in the nonstress treatments. On May 27, 1983, after 9 d of gradually decreasing water supply to the stress treatments, three leaf samplings of plants grown at both 330 and 660 μl/L CO_2 were taken: before sunrise at 0500 EST (dark), at 1100 EST (moderate drought stress), and at 1400 EST (severe drought stress), for both the stressed and unstressed treatments. At each sampling time, 10 uppermost fully expanded leaflets which were completely exposed to sunlight were detached from the remaining plants of each growth chamber and plunged into liquid N_2. The samples were powdered and stored continuously at liquid N_2 temperatures until analysis. This procedure produced no loss of RuBPCase activity or change in activation state over rapidly assayed, fresh material for at least 18 months of storage (29). Immediately after each of the three leaf samplings, 2 whole plants per chamber were sampled for leaf area, fresh weight, and dry weight. Growth analyses before and during this imposed drought stress cycle will be reported elsewhere.

Extraction and Assay of RuBPCase. The extractions and assays of nonactivated (initial) and HCO_3^-/Mg^{2+}-activated RuBPCase were performed in a manner similar to that described previously (30, 31). Kinetic determinations were made on leaf samples of stressed and unstressed plants grown at 330 μl/L CO_2 and harvested at 1400 EST (31). Soluble protein per unit Chl was determined on these samples using methods described before (30). The extraction medium for kinetic measurements was similar to that described before (30), except that 5 mM $MgCl_2$ was included as a precaution against enzyme deactivation. After centrifugation, aliquots of the enzyme extract were applied to a Sephadex G-25 column (1 × 10 cm) which had been equilibrated with 50 mM Tris-HCl buffer containing 0.1 mM EDTA and 5 mM DTT at pH 8.5 and 4°C. The enzyme was eluted with the equilibrating buffer and activated with 5 mM $NaHCO_3$ and 10 mM $MgCl_2$. All assay reactions were performed under N_2 at 30°C in a total volume of 1 ml. RuBPCase activities were expressed on a Chl basis determined by the method of Arnon (1). The CO_2 concentration was calculated as described previously (31) from the pH and HCO_3^- concentration using the Henderson-Hasselbach equation with a pK value of 6.348 at 30°C, and the Henry's law constant for solubility of CO_2 in water at 30°C.

Extraction and Determination of RuBP. RuBP in the liquid N_2-frozen leaf powder was extracted and determined as described previously (29).

Measurement of CO_2 Exchange Rate. Canopy CO_2 exchange rates on a unit ground area basis were measured at 5-min intervals throughout each day during the imposed drought period using the system reported previously (12), and data are reported throughout the day of May 27, 1983 for unstressed and drought stressed chambers under the two CO_2 treatments. Simultaneous measurements of solar PAR (corrected for attenuation by chamber walls) were also taken along with the CER measurements. At the beginning of the drought study on May 18, the diurnal CERs of the two pairs of CO_2 treatments were almost identical with hourly maximum CER values of 19 and 39 $\mu mol/m^2 \cdot s$ for the 330 and 660 μl/L CO_2 treatments, respectively, at an hourly average PAR of 1120 $\mu mol\ quanta/m^2 \cdot s$. The LAI's were 1.11, 1.15, 1.53, and 1.68 m^2 leaf area per m^2 ground area for the 330 μl/L stressed, 330 μl/L unstressed, 660 μl/L stressed, and 660 μl/L unstressed treatments, respectively, on May 18, and most of the floor area of the chamber was covered by vegetation. The canopy CO_2 exchange rates on May 27 were relatively low compared to May 18 values and to previously reported values (12) because of the low number of plants per chamber that remained after a series of removals for whole-plant samples. During the period from dawn to 1100 EST, the leaf area indices (LAI, leaf area per unit ground area) of the plant canopy within the chambers were 0.60, 0.63, 0.89, and 0.88 m^2 leaf area per m^2 ground area for the 330 μl/L stressed, 330 μl/L nonstressed, 660 μl/L stressed, and 660 μl/L nonstressed treatments, respectively. After the 1100 EST whole-plant sampling, only 6, 5, 6, and 5 plants remained in the chambers of the above treatments, respectively, with LAI of 0.45, 0.45, 0.67, and 0.63 m^2 leaf area per m^2 ground area, respectively. The plants that remained on May 27 were not damaged by any of the previous whole-plant samplings. Because of the low density of plants and concomitant low LAI, almost all leaves were exposed to direct-beam solar radiation at some time during the day.

Determination of Leaf Water Potential. Leaf total water potentials were determined on uppermost fully exposed and expanded leaflets from both unstressed and drought stressed plants of the two CO_2 treatments at 1100 EST and 1400 EST on May 27, 1983, using Spanner-type thermocouple psychrometers (26). Duplicate samples were run with four 1-cm diameter leaf discs punched from four different plants for each psychrometer chamber. Osmotic potentials were determined by freezing tissue samples to release cell contents and reduce turgor potential to zero, and redetermination of water potential of these disrupted tissues in the thermocouple psychrometers. Averages of the duplicate samples were computed and leaf turgor potentials were calculated by differences between average leaf total water potentials and average osmotic potentials.

RESULTS

The activities of RuBPCase from leaves of unstressed and drought stressed soybean plants grown at 330 and 660 μl/L CO_2 and sampled at three times during the day of May 27, 1983 are shown in Figure 1, A (initial or nonactivated enzyme) and B (total or HCO_3^-/Mg^{2+}-activated enzyme). On a Chl basis, in all treatments, the nonstressed plants grown at 330 μl/L CO_2 showed slightly more RuBPCase activity (an average of 26% greater) than their counterparts at 660 μl/L CO_2. As we have shown previously for soybean (29), the activity of RuBPCase was substantially lower from leaves sampled before dawn than at 1100 EST, even when the enzyme was activated and assayed at saturating HCO_3^- and Mg^{2+} concentrations. Thus, the predawn-to-

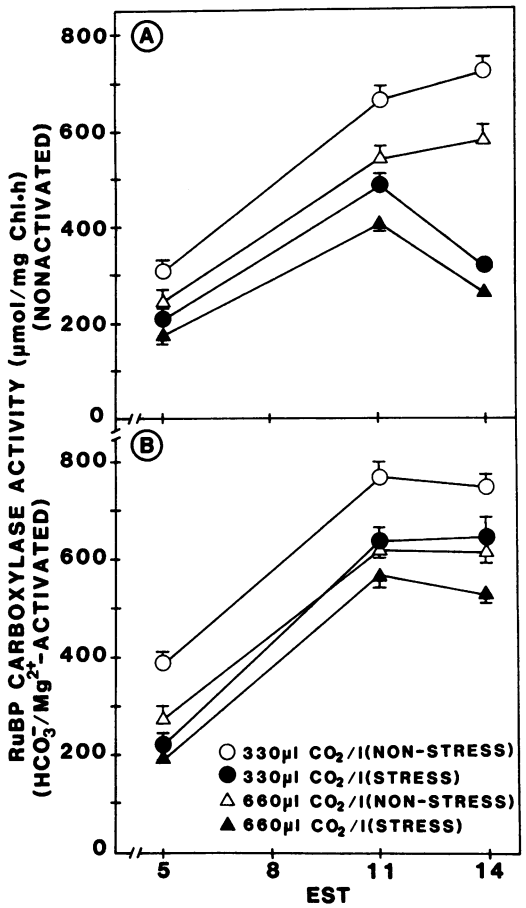

FIG. 1. Diurnal changes in (A) the initial (nonactivated) and (B) total (HCO_3^-/Mg^{2+}-activated) RuBPCase activities in leaf extracts of soybean grown at 330 or 660 μl/L CO_2 and subjected to 0 (nonstress) or 9 d (stress) of gradually increasing drought stress. Error bars denote SD; points lacking error bars indicate that the SD was smaller than the symbols used.

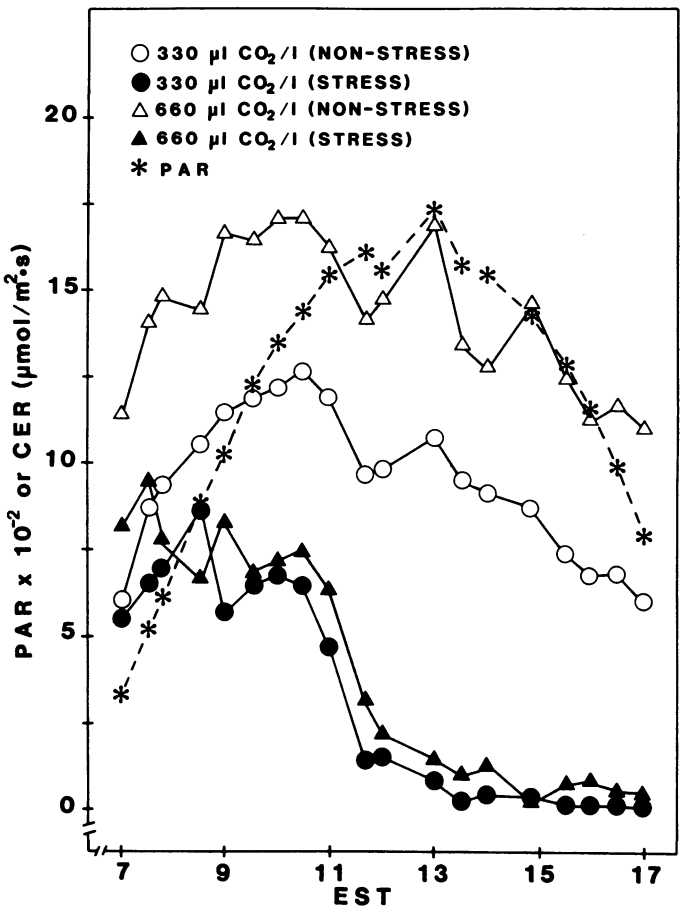

FIG. 2. Diurnal canopy CER for soybeans grown and measured at 330 or 660 μl/L CO_2 and subjected to 0 (nonstress) or 9 d (stress) of gradually increasing drought stress. Measured on May 27, 1983. Solar PAR data are also plotted for reference. CER SD = ±0.75 μmol/m²·s.

1100 EST initial enzyme activity increased by 115 and 117% for the unstressed plants grown at 330 and 660 μl/L CO_2, respectively; and by 132 and 129%, respectively, for the drought stressed plants. The difference between the unstressed and drought stressed treatments was even more apparent when the HCO_3^-/Mg^{2+}-activated enzyme levels were compared (Fig. 1B). The unstressed plants at 330 and 660 μl/L CO_2 showed activity increases of 97 and 125%, respectively, between 0500 and 1100 EST, whereas the corresponding values for the drought stressed plants were 186 and 197%.

At 1400 EST, the initial RuBPCase activities of the drought stressed leaves in both the 330 and 660 μl/L CO_2 treatments were reduced to less than one-half compared to the unstressed plants (Fig. 1A). This occurred, even though the PAR level at 1400 EST was similar to that at 1100 EST (Fig. 2). In contrast to the initial activities, the HCO_3^-/Mg^{2+}-activated RuBPCase values exhibited little or no decrease at 1400 EST even in the drought stressed treatments (Fig. 1B).

Table I contains data for total water, osmotic, and turgor potentials in the soybean leaves at 1100 and 1400 EST. The drought stressed plants, whether grown at 330 or 660 μl/L CO_2, had lower leaf turgor potentials compared to the corresponding unstressed treatments, and the difference was greater at 1400 than at 1100 EST. The turgor potentials were greater for unstressed plants grown at 660 as compared to 330 μl/L CO_2; whereas the osmotic potentials of the unstressed 660 μl/L CO_2 treatment were slightly more negative. Osmotic potentials were

Table I. *Leaf Total Water Potential, Osmotic Potential, and Turgor Potential of Unstressed and Drought Stressed Soybean Plants Grown at CO_2 Concentrations of 330 and 660 μl/L*

Measurements were made on May 27, 1983, after 9 d of drought stress treatment. Average differences of duplicate samples were 0.33 MPa for total water potential, 0.36 MPa for osmotic potential, and 0.07 MPa for turgor potential.

Treatment	Time	Total Water Potential	Osmotic Potential	Turgor Potential
	EST		*MPa*	
330 μl/L CO_2	1100	−1.75	−2.22	0.47
Unstressed	1400	−1.73	−2.19	0.46
330 μl/L CO_2	1100	−1.78	−2.08	0.30
Drought stressed	1400	−2.13	−2.27	0.14
660 μl/L CO_2	1100	−1.65	−2.42	0.77
Unstressed	1400	−1.63	−2.45	0.82
660 μl/L CO_2	1100	−1.69	−2.15	0.46
Drought stressed	1400	−2.07	−2.29	0.22

high; low pod loads may have contributed via low soluble photoassimilate demands on the plant, or high fertilizer salt uptake may have contributed by increasing this source of osmoticum.

To determine if any correlations between RuBPCase activities and net photosynthetic rates might exist, canopy CERs of the different treatments were monitored throughout the day of May

27, 1983. Figure 2 shows the canopy CER data, expressed on a unit chamber ground area basis, for unstressed and drought stressed plants grown at both 330 and 660 μl/L CO_2 levels. At the same PAR level, the diurnal CER of soybeans grown and measured at 660 μl/L CO_2 was substantially higher than that of plants grown and measured at 330 μl/L CO_2, although this difference between the CO_2 treatments was almost eliminated by the imposed drought stress (Fig. 2). Throughout the day of May 27, the overall canopy CER of the unstressed plants was about 50% higher for the plants grown at 660 as compared to those at 330 μl/L CO_2. Before 1100 EST, the canopy CER levels of the stressed plants at both CO_2 concentrations were about 50 to 60% of those of the unstressed plants. However, by 1400 EST, when the diurnal water-stress was most severe, the canopy CER values of the stressed plants had declined to less than 10% of those of the unstressed plants (Fig. 2). This dramatic canopy CER decline to near zero for the stressed plants occurred largely in only 1 h, between 1055 and 1200 EST.

Double reciprocal plots of the activities of RuBPCase in Sephadex-filtered extracts from unstressed and drought stressed soybean leaves, as a function of $H^{14}CO_3^-$ concentration, are shown in Figure 3. These plants were grown at 330 μl/L CO_2, and the leaves were harvested at 1400 EST, when the drought stress on the plants was most severe. The V_{max} values of the RuBPCase from the unstressed plants were somewhat higher than those of the drought stressed plants (Fig. 3). The apparent $K_m(HCO_3^-)$ values in extracts from the unstressed and stressed plants were 1.9 and 2.2 mM, respectively (Fig. 3); while the corresponding, calculated $K_m(CO_2)$ values were 12.6 and 15.0 μM. Saturation by HCO_3^- occurred at between 10 and 15 mM for both extracts. Soluble protein to Chl ratios were similar; 15.2 and 15.5 mg protein per mg Chl for stressed and unstressed samples, respectively.

RuBP levels in leaf samples harvested on May 27 are shown in Figure 4. The RuBP of unstressed and stressed leaves increased from a dark level of about 1 nmol/mg Chl at 0500 EST to between 150 and 200 nmol/mg Chl at 1100 EST, at which time the RuBP levels were 20 to 30% lower in drought stressed leaves than in unstressed leaves. However, at 1400 EST when the drought stress was most severe, and the initial RuBPCase activity

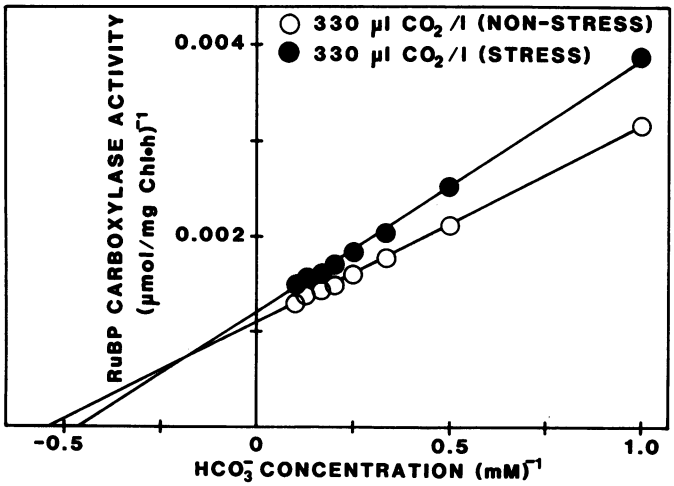

FIG. 3. Double reciprocal plots of total RuBPCase activity as a function of HCO_3^- concentration in leaf extracts of soybeans subjected to 0 (nonstress) or 9 d (stress) of gradually increasing drought stress. The leaves were sampled at 1400 EST on May 27, 1983 from plants grown at 330 μl/L CO_2. Linear regression gave the following equations:

Nonstress	$Y = 0.002059X + 0.001103$	($r^2 = 0.996$)
Stress	$Y = 0.002673X + 0.001205$	($r^2 = 0.999$)

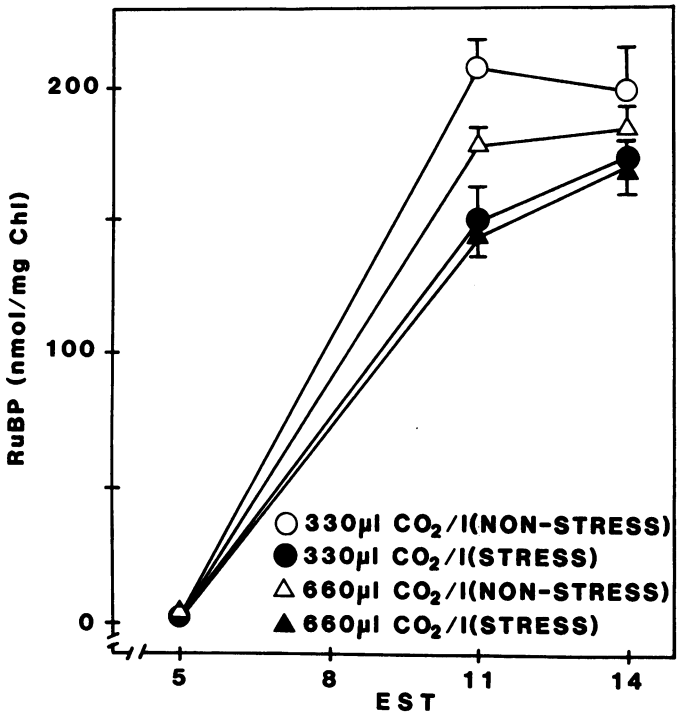

FIG. 4. Diurnal changes in the RuBP levels of leaves from soybeans grown at 330 or 660 μl/L CO_2 and subjected to 0 (nonstress) or 9 d (stress) of gradually increasing drought stress. Error bars denote SD; points lacking error bars indicate that the SD was smaller than the symbols used.

(Fig. 1A) and canopy CER (Fig. 2) of stressed plants had declined, the RuBP levels in the drought stressed leaves increased somewhat to values that were only about 8 to 15% less than the unstressed leaf levels. On a Chl basis, the RuBP levels during the day in leaves grown at ambient CO_2 levels were higher than in leaves grown at enriched-CO_2, but these differences were almost eliminated by the drought stress treatment.

DISCUSSION

It is apparent that mild drought stress does not appreciably affect extractable RuBPCase activity (10, 18, 19, 27), although exceptions to this have been reported for some plants, including cotton and bean leaves (11, 20). Moderate to severe drought stress, especially over longer time periods, does alter RuBPCase activity (18, 19, 27, 28). However, the contribution of this nonstomatal component to the overall reduction in CER, as compared with that of stomatal closure, is subject to debate (6, 8, 24), and may differ as a function of the CO_2 level (6).

From our diurnal data, two distinct but related effects of drought stress on soybean RuBPCase activity were observable. The first was a chronic effect, in which the stressed plants exhibited less RuBPCase activity than the unstressed controls. This occurred irrespective of the time of day the enzyme was assayed, and whether or not it was activated with HCO_3^-/Mg^{2+} after extraction. Also, growth under the enriched-CO_2 atmosphere served only to exacerbate this effect. This reduction in RuBPCase activity may be due to decreased synthesis (11) and/or increased degradation (7, 22) of soluble protein. However, in willow leaves the reduction in RuBPCase activity under severe drought stress was not due to loss of RuBPCase protein, but may have been influenced by the relative proportions of the enzyme in crystalline and soluble forms (28). Soluble protein levels in the stressed and unstressed 330 μl/L CO_2 leaves were similar. There was no indication that the long-term drought stress effects on RuBPCase were due to changes in the K_m of the enzyme.

The second effect was more transient and occurred as the drought stress became most severe during the afternoon hours. It was characterized by a substantial decline between 1100 and 1400 EST in the initial (nonactivated) RuBPCase activities of the drought stressed plants grown in ambient or enriched CO_2. This afternoon decline was largely recoverable by *in vitro* activation with HCO_3^-/Mg^{2+}, suggesting that the *in vivo* activation of the enzyme was decreased by the increasing drought stress. Even at 1100 EST, before the stress became most acute, the initial RuBPCase activities of the stressed plants were only 71 to 77% of the total (fully activated), whereas corresponding values for the unstressed plants were 87 to 88%.

Recently it has been demonstrated with illuminated, intact chloroplasts and leaf slices that induced osmotic stress results in acidification of the stroma (3, 5), with pH values dropping to near levels found in the dark. Furthermore, the data (4) suggest that activity of RuBPCase could be affected by stromal acidification. Lorimer *et al.* (16), working with purified spinach RuBPCase, have shown that in the presence of 10 μM CO_2 and 20 mM Mg^{2+}, activation of this enzyme increases more than 4-fold with a pH change from 7.5 to 8.5. Stromal acidification under moderate to severe drought stress, producing a less activated enzyme, could explain not only the decline of *in vivo* CO_2 fixation catalyzed by RuBPCase, but also the transient decline of initial *in vitro* activities observed with the extracted soybean enzyme.

The transient decline in initial activity could not be due to the continued presence in high light of the 'dark' inhibitor of RuBPCase we have described in soybean leaves (29), because the total activity was easily recoverable with HCO_3^-/Mg^{2+}-activation. However, there was indication that long-term drought stress increased the degree to which the inhibitor influenced the enzyme, as shown by the greater difference between the dark (0500 EST) and light (1100 EST) total activity determinations for drought stressed, as compared with unstressed, plants.

The diurnal canopy CER curves for unstressed plants tended to track the solar PAR, although there was some deviation in the morning hours for the enriched-CO_2 treatment, which exhibited quite high net photosynthetic rates while the quantum irradiance was still low. However, the quantum yield at 0700 EST for the unstressed 660 μl/L CO_2 treatment was only 0.035 mol CO_2/mol quanta (ground area basis) or 0.040 mol CO_2/mol quanta (leaf area basis) which is reasonable for this level of CO_2 even with sparse plants. The CER of the stressed treatments did not track the solar PAR during the morning but remained nearly constant, which indicates a gradual increase in drought stress effects. Except for the early morning values, the drought stressed plants showed substantially lower canopy CERs than either of the unstressed treatments, and starting about 1055 EST there was a precipitous decline, which within about 1 h gave values approaching zero. These low values continued for the remainder of the day, even though solar PAR was high. It is notable that the canopy CERs for the enriched- and ambient-CO_2 drought stressed treatments were very similar throughout the day. Thus, in this case, CO_2 enrichment did not appear capable of alleviating moderate to severe drought stress effects on soybean canopy photosynthetic rates. The drought stress virtually eliminated, during the stress period, any beneficial effects of CO_2-enrichment on soybean photosynthesis. This contrasts with yield data for pea and wheat which were increased by CO_2-enrichment, despite a period of drought stress (21, 25). However, in these two studies the parameter measured, yield, was probably influenced by photosynthesis during the unstressed, as well as during the stressed period.

The maximum canopy CER values on May 27 were considerably lower than reported previously (12) because plant density and, concomitantly, LAI was much lower since many of the plants had been removed for growth analyses as a part of another experiment. The maximum CER values for unstressed treatments were only about 17 and 12 μmol/(m^2·s) for 660 and 330 μl/L CO_2 treatments, respectively, during the morning of May 27. However, the LAI values were only 0.88 and 0.63 for those respective treatments, and the leaf area was clumped around individual plants rather than distributed throughout the chamber. The ratio of maximum CER (17/12 = 1.4) for 660 *versus* 330 μl/L CO_2 treatments was slightly smaller than the ratio (90/60 = 1.5) for nonstressed full-canopy 800 *versus* 330 μl/L CO_2 treatments reported earlier (12). Therefore, the ratios of canopy CER responses to CO_2 enrichment under nonstressed conditions are similar to previous observations, and overall low values of canopy CER resulted solely because of low LAI.

The diurnal canopy CER and the RuBPCase initial activity curves had a number of features in common, including lower values for drought stressed, than unstressed, treatments. Also, due to the presence of the soybean RuBPCase 'dark' inhibitor (29), the early morning values for RuBPCase activity and canopy CER were lower than later in the day, when solar PAR was high. Even the rapid decline in canopy CER of the drought stressed plants after 1100 EST was reflected by a decline in the initial RuBPCase activities. Whether the decreased activation of RuBPCase initiated the rapid reduction in canopy CER, or followed after stomatal closure commenced, cannot be determined from these data. For *Sinapis alba* subjected to rapid and severe drought stress, Cornic *et al.* (6) have reported that nonstomatal effects were the major initial cause of the decrease in assimilation rate, especially at ambient and twice-ambient CO_2 concentrations. In the present study, the decline in initial RuBPCase activities after 1100 EST cannot explain all of the reduction in canopy CER during the afternoon hours, because the carboxylase values were only halved by the drought stress, whereas the canopy rates dropped to near zero. Furthermore, prior to the onset of the acute stress, the differences in canopy rates for stressed and unstressed plants were much greater than the comparable differences in RuBPCase activity values. However, it is clear that moderate to severe drought stress may affect not only the amount of RuBPCase protein but also the activation state of the enzyme. This latter could well play an important and rapid role in the nonstomatal responses of plants to drought stress.

Since only the uppermost, fully expanded leaves were sampled, we cannot say with certainty what the RuBPCase response to CO_2 and drought stress throughout the whole canopy would have been. However, because of the low plant density and low LAI, most of the leaves received direct sunlight at least part of the day. Therefore, we expect that our sample was representative of the whole canopy in our experiment.

Despite the lower RuBPCase activities in stressed leaves, the daytime RuBP concentrations were also less in the stressed treatments, suggesting that regeneration of RuBP was affected by the drought stress. This was true irrespective of the CO_2 treatment. A similar reduction of the RuBP regeneration capacity by drought stress was reported in isolated mesophyll cells of *Xanthium strumarium* (24), and in spinach chloroplasts (4), due to drought stress effects on photophosphorylation, and/or P-ribulokinase (4, 24). It is interesting that when the most severe drought stress occurred in the afternoon, the RuBP levels in soybean leaves actually rose slightly. This may indicate that RuBP regeneration in soybean is less affected by drought stress than other nonstomatal events, or that utilization of RuBP for CO_2 fixation was so low that it did not deplete the RuBP pool. In either case, it is evident that RuBP levels were probably not a factor in these drought stress limitations to soybean leaf photosynthesis.

Acknowledgments—The authors thank Dr. Pierce Jones for maintaining the controlled-environment chambers used for collecting photosynthetic data, P. R. Harris (deceased) for measurements of leaf water potential, M. DeMaio and W. W.

Wynn for construction of pots and preparation of rooting medium (including calculation of water-holding capacity), and W. J. Campbell and P. R. Harris for differential irrigation management during the drying cycle.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. BAES CF, HE GOELLER, JC OLSON, RM ROTTY 1977 Carbon dioxide and climate: the uncontrolled experiment. Am Sci 65: 310–320
3. BERKOWITZ GA, M GIBBS 1983 Reduced osmotic potential effects on photosynthesis. Identification of stromal acidification as a mediating factor. Plant Physiol 71: 905–911
4. BERKOWITZ GA, M GIBBS 1983 Reduced osmotic potential inhibition of photosynthesis. Site-specific effects of osmotically induced stromal acidification. Plant Physiol 72: 1100–1109
5. BERKOWITZ GA, C CHEN, M GIBBS 1983 Stromal acidification mediates *in vivo* water stress inhibition of nonstomatal-controlled photosynthesis. Plant Physiol 72: 1123–1126
6. CORNIC G, J-L PRIOUL, G LOUASON 1983 Stomatal and non-stomatal contribution in the decline in leaf net CO_2 uptake during rapid water stress. Physiol Plant 58: 295–301
7. DWIVEDI S, M KAR, D MISHRA 1979 Biochemical changes in excised leaves of *Oryza sativa* subjected to water stress. Physiol Plant 45: 35–40
8. FARQUHAR GD, TD SHARKEY 1982 Stomatal conductance and photosynthesis. Annu Rev Plant Physiol 33: 317–345
9. FELLOWS RJ, JS BOYER 1976 Structure and activity of chloroplasts of sunflower leaves having various water potentials. Planta 132: 229–239
10. HUFFAKER RC, T RADIN, GE KLEINKOPF, EL COX 1970 Effects of mild water stress on enzyme of nitrate assimilation and of the carboxylative phase of photosynthesis in barley. Crop Sci 10: 471–474
11. JONES HG 1973 Moderate-term water stresses and associated changes in some photosynthetic parameters in cotton. New Phytol 72: 1095–1105
12. JONES P, LH ALLEN JR, JW JONES, KJ BOOTE, WJ CAMPBELL 1984 Soybean canopy growth, photosynthesis, and transpiration responses to whole-season carbon dioxide enrichment. Agron J 76: 633–637
13. KAISER WM, U HEBER 1981 Photosynthesis under osmotic stress. Effect of high solute concentrations on the permeability properties of the chloroplast envelope and on activity of stroma enzymes. Planta 153: 423–429
14. KECK RW, JS BOYER 1974 Chloroplast response to low leaf water potentials. III. Differing inhibition of electron transport and photophosphorylation. Plant Physiol 53: 474–479
15. LAWLOR DW 1976 Water stress induced changes in photosynthesis, photorespiration, respiration and CO_2 compensation concentration of wheat. Photosynthetica 10: 378–387
16. LORIMER GH, MR BADGER, TJ ANDREWS 1977 D-Ribulose-1,5-bisphosphate carboxylase-oxygenase. Improved methods for activation and assay of catalytic activities. Anal Biochem 78: 66–75
17. MANABE S, RT WETHERALD 1980 On the distribution of climate change resulting from an increase in CO_2-content of the atmosphere. J Atmos Sci 37: 99–118
18. MAYORAL ML, D ATSMON, D SHIMSHI, Z GROMET-ELHANAN 1981 Effect of water stress on enzyme activities in wheat and related wild species: carboxylase activity, electron transport and photophosphorylation in isolated chloroplasts. Aust J Plant Physiol 8: 385–393
19. O'TOOLE JC, RK CROOKSTON, KJ TREHARNE, JL OZBUN 1976 Mesophyll resistance and carboxylase activity. A comparison under water stress conditions. Plant Physiol 57: 465–468
20. O'TOOLE JC, JL OZBUN, DH WALLACE 1977 Photosynthetic response to water stress in *Phaseolus vulgaris*. Physiol Plant 40: 111–114
21. PAEZ A, H HELLMERS, B STRAIN 1983 CO_2 enrichment, drought stress and growth of Alaska pea plants (*Pisum sativum*). Physiol Plant 58: 161–165
22. PEOPLES MB, MJ DALLING 1978 Degradation of ribulose-1,5-bisphosphate carboxylase by proteolytic enzymes from crude extracts of wheat leaves. Planta 138: 153–160
23. ROGERS HH, JF THOMAS, GE BINGHAM 1983 Response of agronomic and forest species to elevated atmospheric carbon dioxide. Science 220: 428–429
24. SHARKEY TD, MR BADGER 1982 Effects of water stress on photosynthetic electron transport, photophosphorylation, and metabolite levels of *Xanthium strumarium* mesophyll cells. Planta 156: 199–206
25. SIONIT N, H HELLMERS, BR STRAIN 1980 Growth and yield of wheat under CO_2 enrichment and water stress. Crop Sci 20: 687–690
26. SPANNER DC 1951 The Peltier effect and its use in the measurement of suction pressure. J Exp Bot 2: 145–168
27. VAPAAVUORI EM, NKS VALANNE 1982 Activities of ribulose-1,5-bisphosphate carboxylase-oxygenase in *Salix* sp. during water stress. Photosynthetica 16: 1–6
28. VAPAAVUORI EM 1986 Correlation of activity and amount of ribulose-1,5-bisphosphate carboxylase with chloroplast stroma crystals in water-stressed willow leaves. J Exp Bot 37: 89–98
29. VU CV, LH ALLEN JR, G BOWES 1983 Effects of light and elevated atmospheric CO_2 on the ribulose bisphosphate carboxylase activity and ribulose bisphosphate level of soybean leaves. Plant Physiol 73: 729–734
30. VU JCV, LH ALLEN JR, G BOWES 1984 Dark/light modulation of ribulose bisphosphate carboxylase activity in plants from different photosynthetic categories. Plant Physiol 76: 843–845
31. VU JCV, G BOWES, LH ALLEN JR 1986 Properties of ribulose-1,5-bisphosphate carboxylase from dark- and light-exposed soybean leaves. Plant Sci 44: 119–123