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Effects of Light and Elevated Atmospheric CO_2 on the Ribulose Bisphosphate Carboxylase Activity and Ribulose Bisphosphate Level of Soybean Leaves[1]

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ABSTRACT

Soybean (*Glycine max* L. Merr. cv Bragg) was grown throughout its life cycle at 330, 450, and 800 microliters CO_2 per liter in outdoor controlled-environment chambers under solar irradiance. Leaf ribulose-1,5-bisphosphate carboxylase (RuBPCase) activities and ribulose-1,5-bisphosphate (RuBP) levels were measured at selected times after planting. Growth under the high CO_2 levels reduced the extractable RuBPCase activity by up to 22%, but increased the daytime RuBP levels by up to 20%.

Diurnal measurements of RuBPCase (expressed in micromoles CO_2 per milligram chlorophyll per hour) showed that the enzyme values were low (230) when sampled before sunrise, even when activated *in vitro* with saturating HCO_3^- and Mg^{2+}, but increased to 590 during the day as the solar quantum irradiance (photosynthetically active radiation or PAR, in micromoles per square meter per second) rose to 600. The nonactivated RuBPCase values, which averaged 20% lower than the corresponding HCO_3^- and Mg^{2+}-activated values, increased in a similar manner with increasing solar PAR. The per cent RuBPCase activation (the ratio of nonactivated to maximum-activated values) increased from 40% before dawn to 80% during the day. Leaf RuBP levels (expressed in nanomoles per milligram chlorophyll) were close to zero before sunrise but increased to a maximum of 220 as the solar PAR rose beyond 1200. In a chamber kept dark throughout the morning, leaf RuBPCase activities and RuBP levels remained at the predawn values. Upon removal of the cover at noon, the HCO_3^- and Mg^{2+}-activated RuBPCase values and the RuBP levels rose to 465 and 122, respectively, after only 5 minutes of leaf exposure to solar PAR at 1500.

These results indicate that, in soybean leaves, light may exert a regulatory effect on extractable RuBPCase in addition to the well-established activation by CO_2 and Mg^{2+}.

The global atmospheric CO_2, presently at about 340 μl CO_2/l, is increasing at an annual rate greater than 1 μl CO_2/l (3). Current estimates predict that the atmospheric CO_2 concentration could double before the end of the next century. Short-term effects of elevated CO_2 on photosynthesis are well documented (12). However, it is only recently that studies have been initiated on long-term growth effects under moderately increased CO_2 levels, such as reasonably could be expected to occur within the foreseeable future. Since the current CO_2 level does not saturate C_3 photosynthesis (9, 12), and thus the growth of most crop plants, the predicted increases in the ambient CO_2 level could potentially have a beneficial effect on crop productivity (1, 29).

RuBPCase[2] is the enzyme responsible for the fixation of atmospheric CO_2 into the PCR cycle of C_3 plants. Because of its potential rate-limiting role in photosynthesis and photorespiration (18), this enzyme has been subject to intensive study in the past decade, but its activation and regulation *in vivo* have yet to be fully defined. With intact, isolated chloroplasts, intact wheat leaves and *Arabidopsis* plants, the activity of RuBPCase has been shown to be dependent upon both CO_2 and light (4, 16, 23). Light appears to have an indirect effect *in vivo* in that it causes an increase in pH and Mg^{2+} levels in the chloroplast stroma (21, 27). It is now well established that *in vitro* Mg^{2+} and CO_2 act as activators of RuBPCase (15). Various chloroplast metabolites also have been shown either to positively or negatively influence RuBPCase activity *in vitro* (5–7, 10, 17), though, *in vivo*, the precise role, if any, of these compounds in the regulation of the enzyme is still obscure.

Although the CO_2 assimilation rate of a C_3 leaf has been correlated with the activity of RuBPCase (18), it has also been suggested that at high intercellular CO_2 levels the rate of RuBP regeneration may become limiting (25). Recently, it has been reported that the RuBP levels in wheat leaves were dependent upon the leaf irradiance prior to extraction (19). Thus, both CO_2 and light may play important roles in regulating RuBP levels in C_3 leaves, as well as determining the degree of RuBPCase activation.

In this study, soybeans were grown throughout their life cycle under natural solar irradiance in controlled-environment chambers with atmospheric CO_2 levels of 330, 450, and 800 μl CO_2/l. The effects of elevated CO_2 levels on extractable RuBPCase activity and RuBP levels are examined. Furthermore, we report on substantial, light-induced variations in extractable RuBPCase activity from soybean leaves, which appear not to be mediated through the established HCO_3^-/Mg^{2+}-activation mechanism.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Soybeans (*Glycine max* L. Merr. cv Bragg) were planted on August 13, 1982, and grown in Plexiglas controlled-environment growth chambers located outdoors in Gainesville, FL, so that the plants received

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[2] Abbreviations: RuBPCase, ribulose-1,5-bisphosphate carboxylase-oxygenase; PCR, photosynthetic carbon reduction; RuBP, ribulose-1,5-bisphosphate; EST, eastern standard time.

direct, natural solar irradiance (11). These chambers are similar to the Soil-Plant-Atmosphere Research (SPAR) units developed by Phene *et al.* (20). The soybean plants were grown throughout their life cycle at three different CO_2 concentrations: 330 μl/l (two chambers), 450 μl/l (one chamber), and 800 μl/l (two chambers). Each chamber enclosed 2 m^2 of ground area and contained four 50-cm rows, nine plants/row, resulting in a total of 36 plants/chamber. Lysimeters or soil bins (1 × 2 m in area and 1 m deep) provided a large soil volume for plants growing in the chambers. Soil of the SPAR units in which the soybeans were grown was an Arredondo fine sand reconstructed by layers when the bins were filled. Plexiglas chamber tops (1.5 m in height) enclosed the aerial plant environment. Each chamber had an air-conditioning system attached via ductwork to its north side where an access port also was present. Dry bulb temperature, CO_2 concentration, and dewpoint temperature were controlled by digital computer. The dry bulb temperature was controlled at 31 ± 1°C during the day and 23 ± 1°C during the night, and the dewpoint temperature was 21 ± 2°C during the day. The detailed chamber characteristics and the systems for controlling and monitoring environmental parameters in the chambers were described by Jones (11). CO_2 exchange rates and transpiration rates of individual leaves and of the canopy within the SPAR chambers were obtained throughout the season and will be reported fully elsewhere.

The seeds germinated on August 16, 1982. Four incandescent lamps over each chamber provided light from 1700 EST to 2300 EST for 2 weeks after planting to extend the photoperiod and prevent premature flowering.

Leaf Sampling Procedures. On October 5, 1982, 53 d after planting when the plants were at the R-3 growth stage, diurnal samplings of leaves in the 330, 450, and 800 μl/l CO_2 chambers were performed, starting before sunrise (0600 EST) and terminating at 1345 EST. At each sampling time, nine uppermost fully expanded leaflets which were completely exposed to sunlight were detached from nine different plants, and plunged immediately into liquid N_2 and then continuously stored in liquid N_2 until analysis.

On November 1, 1982, when the plants were at the R-6 growth stage, one 330 μl CO_2/l chamber was completely covered at sunset to create artificially dark conditions during the next day. The other, uncovered, 330 μl/l CO_2 chamber served as the control. The covered chamber was monitored to ensure the plants were not exposed to light. On November 2, 1982, 81 d after planting, samplings of the uppermost fully expanded leaflets in these two 330 μl/l CO_2 chambers were performed, starting before sunrise (0600 EST) and ending 1 h after sunset (1900 EST). A total of seven samplings were taken during the day from the control chamber. In the covered chamber, four samplings were made up to 1210 EST. The cover was then removed at 1215 EST and a second set of six samplings were made over the rest of the day. Leaflets taken at each time point were immediately immersed and stored in liquid N_2. The time from detaching the leaflet from the plant to plunging it into liquid N_2 was less than 1 s.

Extraction and Assay of RuBP Carboxylase. Liquid N_2-frozen leaf samples were ground to a powder in liquid N_2 with a mortar and pestle. All subsequent work was performed at 0°C. A portion of the frozen powder (equivalent to approximately 2 mg Chl) was transferred to a pre-chilled Ten Broeck homogenizer and was ground in 10 ml of extraction medium which consisted of 50 mM Tris-HCl, 5 mM DTT, 0.1 mM EDTA, and 1.5% (w/v) PVP-40 at pH 8.5. Aliquots of the homogenates were taken for Chl determinations (2). The remainder was centrifuged for 3 min at 15,000g and the supernatant was immediately used for assays.

Assay reactions were performed at 30°C in a total volume of 1 ml in 5-ml Reacti-Vials[3] (Pierce Chemical Co.) which were sealed with serum stoppers and screw-caps. The reaction mixtures, which consisted of 50 mM Tris-HCl (CO_2-free), 5 mM DTT, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM RuBP, and 20 mM $NaH^{14}CO_3$ (0.2 μCi/μmol) at pH 8.5, were flushed with N_2 for 10 min prior to adding the Tris buffer and $NaH^{14}CO_3$. The initial (nonactivated) RuBPCase activity was measured by injecting 0.1 ml of the supernatant into the assay mixture and terminating the reaction after 45 s with 0.1 ml of 1 N HCl. For measurement of the HCO_3^-/Mg^{2+}-activated RuBPCase, an aliquot of the crude supernatant was incubated with 10 mM $NaHCO_3$ and $MgCl_2$ at room temperature for 10 min prior to assay. After assay, aliquots (0.9 ml) were transferred to scintillation vials and dried in an airstream. The acid-stable ^{14}C radioactivity was then determined by liquid scintillation spectrometry. No differences in RuBPCase activities between fresh and liquid N_2-frozen samples were observed, even after storage of the samples for 8 months in liquid N_2.

Extraction and Determination of RuBP. RuBP in the liquid N_2-frozen leaf powder was extracted and determined by modification of the methods described by Créach and Stewart (8) and Latzko and Gibbs (14). Frozen leaf powder (equivalent to approximately 3 mg Chl) was ground in a Ten Broeck tissue homogenizer containing 10 ml of 0.5 N HCl at 0°C. An aliquot was used for Chl determination. The remainder of the homogenate was centrifuged for 5 min at 8000g. A 5-ml aliquot of the supernatant was brought to pH 8.3 by the addition of 0.75 ml 2 M Tris base and 0.44 ml 4 N KOH. RuBP was then assayed using crystalline RuBPCase prepared from field-grown leaves of tobacco (*Nicotiana tabacum* cv NC2512) as described by Kung *et al.* (13). The assays were carried out at 30°C in a total volume of 1 ml in serum-stoppered Reacti-Vials which contained 0.4 ml of 50 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 5 mM DTT, 20 mM $NaH^{14}CO_3$ (0.2 μCi/μmol), and a 0.5-ml aliquot of the supernatant leaf extract. The reaction was initiated with 0.1 ml of the tobacco RuBPCase (equivalent to 0.15 mg protein) which had been previously dissolved in 100 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 10 mM $NaHCO_3$, and 100 mM NaCl, and reactivated at 50°C for 25 min (13). After 1 h, the reaction was terminated with 0.1 ml 1 N HCl. Aliquots (0.9 ml) were then dried in an airstream and their radioactivity was determined by liquid scintillation spectrometry. The RuBP levels in the liquid N_2-frozen leaf tissue samples were found to remain unchanged for at least 8 months of storage.

Since Chl was converted to pheophytin during the extraction of leaf powder in dilute HCl, the original Chl concentration was determined by measuring pheophytin (24). Separate determinations showed that this procedure gave values for Chl within 3% of that obtained by the method of Arnon (2).

Both RuBPCase activities and RuBP concentrations were expressed on a Chl basis. Each data point represents the mean value of triplicate determinations from each of two subsamples from the combined pool of nine leaflets.

RESULTS

Diurnal Changes in RuBPCase Activity. Figure 1 shows the activity of RuBPCase from soybean leaves harvested on October 5, 1982 (an overcast day) at various times during the day. The data show the enzyme activity for RuBPCase in the HCO_3^-/Mg^{2+}-activated and nonactivated states, and the PAR level at the time the leaves were harvested. The plants were grown from seed under three different CO_2 levels. The activity of RuBPCase was

[3] Mention of a trademark name of a proprietary product in this paper does not imply endorsement by the University of Florida, the United States Department of Agriculture, or the United States Department of Energy.

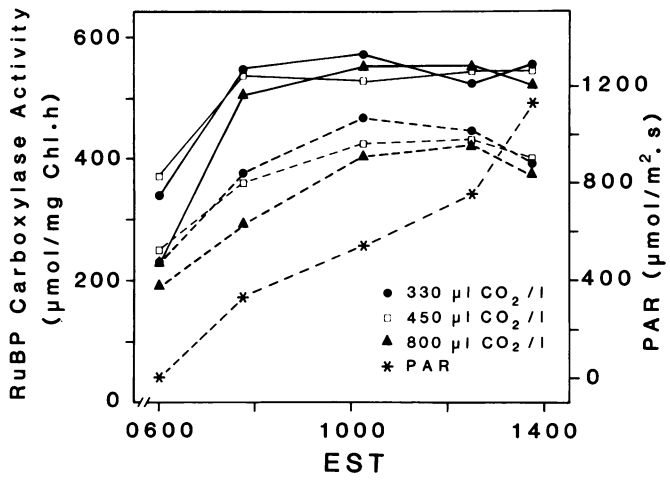

FIG. 1. Diurnal changes in the HCO_3^-/Mg^{2+}-activated (——) and nonactivated (- - -) RuBPCase values from leaves of 53-d-old soybean plants grown under three CO_2 concentrations (330, 450, and 800 μl CO_2/l). The leaf samples were harvested, at the time shown, on October 5, 1982. The PAR at the time of sampling is plotted for reference.

Table I. *HCO_3^-/Mg^{2+}-Activated RuBPCase Values from Leaves of Soybean Grown under Three CO_2 Concentrations*

The enzyme activities were determined on leaves sampled at 0830 EST, 21 and 39 d after the planting date of August 13, 1982. At 21 d, the leaves were expanding; at 39 d, the leaves were fully expanded.

CO_2 Treatment	RuBPCase Activity	
	21 d	39 d
μl CO_2/l	*μmol CO_2/mg Chl·h*	
330	377.3 ± 18.5[a]	558.7 ± 7.9
450	358.8 ± 17.5	512.2 ± 10.7
800	290.8 ± 13.0	447.5 ± 11.9

[a] Mean ± SE.

substantially lower from leaves harvested in the early morning when the PAR was zero, even when the enzyme was activated and assayed at saturating HCO_3^- and Mg^{2+} concentrations. This was true regardless of the CO_2 level used for growth. Thus, the increases in the HCO_3^-/Mg^{2+}-activated RuBPCase values measured at 0745 EST, as compared to 0600 EST, were 61, 46, and 123% for the 330, 450, and 800 μl/l CO_2 growth treatments, respectively. After 0745 EST, when the PAR was 330 μmol/m²·s or higher, the extractable RuBPCase activity remained relatively constant. Similar trends were observed with the nonactivated enzyme, in that the RuBPCase activity from all CO_2 treatments was approximately doubled at 1015 EST (when the PAR was 540 μmol/m²·s) as compared with 0600 EST. In all three CO_2 treatments, the value of the nonactivated enzyme was always lower than the corresponding HCO_3^-/Mg^{2+} activated value (Fig. 1).

Some differences between the control CO_2 treatment (330 μl CO_2/l) and the highest CO_2 growth treatment (800 μl CO_2/l) in both the HCO_3^-/Mg^{2+}-activated and nonactivated RuBPCase values also were observed (Fig. 1). At 0600 EST, the HCO_3^-/Mg^{2+}-activated enzyme of the 330 μl CO_2/l control was 50% higher than that of the 800 μl CO_2/l treatment. At other sampling times during the day, the differences between these two CO_2 treatments were not significant. However, the nonactivated RuBPCase values from the highest CO_2 growth treatment were consistently lower than those of the 330 μl CO_2/l control, and this trend was more marked during the morning sampling periods (Fig. 1).

Further determinations of the HCO_3^-/Mg^{2+}-activated RuBPCase from plants grown under the three CO_2 levels, but at earlier growth stages, are presented in Table I. These data confirm that RuBPCase activity was lowest in leaf extracts of the 800 μl CO_2/l treatment. When compared to the 330 μl CO_2/l control, enzyme activities were 77 and 95% for the 800 and 450 μl CO_2/l treatments, respectively, at 21 d (V-2 growth stage, expanding leaves) after planting; and the corresponding values at 39 d (V-8 growth stage, fully expanded leaves) after planting were 80 and 92%.

On November 2, 1982, while one 330 μl CO_2/l chamber served as the control, the second 330 μl CO_2/l chamber was completely covered to extend the dark period throughout the morning until 1215 EST. RuBPCase activities for leaves from both the covered and uncovered plants sampled throughout that day are shown in Figure 2A (nonactivated enzyme) and Figure 2B (HCO_3^-/Mg^{2+}-activated enzyme). In the uncovered control plants, RuBPCase values for both the HCO_3^-/Mg^{2+}-activated and nonactivated state increased after sunrise to reach their maximum at 1055 EST, when the PAR was 800 μmol/m²·s. These maximum values remained unchanged for at least 4 h, as solar PAR continued to increase to 1500 and then declined to 600 μmol/m²·s, at 1510 EST. As the solar PAR dropped below 600 μmol/m²·s during the late afternoon, the enzyme activities declined and after sunset (1900 EST) they approached similar values to those measured at 0600 EST. In contrast to the data for control plants, RuBPCase activities from leaves in the covered (dark) chamber remained at a constant low level throughout the morning, irrespective of

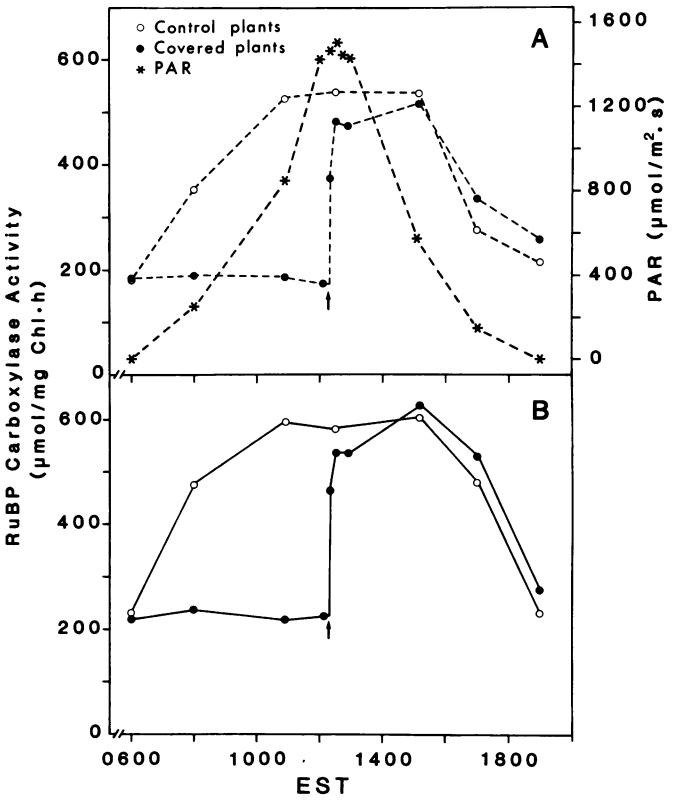

FIG. 2. Diurnal changes in nonactivated (A) and HCO_3^-/Mg^{2+}-activated (B) RuBPCase values from leaves of 81-d-old soybean plants grown at 330 μl CO_2/l. The control plants were exposed to natural solar irradiance throughout the day. The covered plants were kept dark until noon, at which time the chamber cover was removed to expose the plants to solar irradiance (arrows). The leaf samples were harvested, at the time shown, on November 2, 1982. The PAR at the time of sampling is plotted for reference in A.

whether or not the enzyme was activated *in vitro* with HCO_3^- and Mg^{2+} (Fig. 2, A and B). However, when the cover was removed at 1215 EST and leaf samples were harvested at 1220 EST and thereafter, a rapid dark-to-light transition effect on the extracted RuBPCase activity was very obvious (Fig. 2, A and B). The activity of RuBPCase sharply increased; HCO_3^-/Mg^{2+}-activated and nonactivated RuBPCase values at 1220 EST increased by at least 2-fold as compared to those measured prior to 1220 EST. The activities continued to increase and reached maximum values similar to those of the control, at which point they were almost 3-fold higher than those of dark leaves. Both HCO_3^-/Mg^{2+}-activated and nonactivated values began to decrease and followed the patterns of those of the control when the PAR declined in the late afternoon. Although both HCO_3^-/Mg^{2+}-activated and nonactivated values followed the same general patterns throughout the day, the nonactivated values were always lower than the HCO_3^-/Mg^{2+}-activated in both the control and covered chambers.

In Figure 3, HCO_3^-/Mg^{2+}-activated RuBPCase values and the per cent activation from leaves of the control treatment (330 μl CO_2/l), determined at two different sampling dates (Oct. 5 and Nov. 2, 1982), are plotted as a function of solar PAR. The RuBPCase activity, even when activated *in vitro* with saturating HCO_3^- and Mg^{2+}, was low when the PAR was zero but it doubled as the solar PAR increased to 600 μmol/m^2·s (Fig. 3, upper curve). Further increases in PAR did not increase the activity of the enzyme. Per cent activation values on each day were computed as the percentages of the ratio of nonactivated values to the maximum activated values for each day (Fig. 3, lower curve). These data (Fig. 3) demonstrate that RuBPCase activation, as compared with the potential maximum, was low in the dark but increased as solar PAR increased, and reached light saturation at about 600 μmol/m^2·s.

Diurnal Changes in RuBP Levels. Diurnal RuBP levels in leaf samples harvested on October 5, 1982, are shown in Figure 4. At 0600 EST when the solar PAR was zero, only about 1 nmol RuBP/mg Chl was detected in the soybean leaves. However, at 0745 EST when the solar PAR was about 330 μmol/m^2·s, large increases in the RuBP level for all three CO_2 growth treatments were evident. Further increases in RuBP with increased solar PAR were much more gradual. The daytime RuBP levels in leaves of soybean plants grown under the enriched CO_2 conditions were higher than in leaves of control plants grown at 330 μl CO_2/l (Fig. 4).

Figure 5 shows the diurnal patterns of RuBP levels in soybean leaves from the uncovered control chamber and the covered chamber in which the dark period was extended until 1215 EST on November 2, 1982. In the uncovered control, the RuBP increased from its dark level of 1.2 nmol/mg Chl at 0600 EST to about 120 nmol/mg Chl at 0800 EST when solar PAR was 250 μmol/m^2·s (Fig. 5). The RuBP exhibited a further increase to about 220 nmol/mg Chl after the 1055 EST sampling time, when the PAR was 800 μmol/m^2·s, at which level it remained constant until the PAR declined in the late afternoon.

In the covered chamber, leaf RuBP levels were maintained at about 0.9 nmol/mg Chl as long as the plants were kept in the dark conditions. However, when the cover was removed from the chamber at noon and the plants were exposed to solar PAR at 1500 μmol/m^2·s, a sharp increase in RuBP levels to 120 nmol/mg Chl was observed after 5 min of exposure to the sunlight (Fig. 5). Unlike the control, the RuBP level in leaves from the covered chamber remained constant at 120 nmol/mg Chl, even after several hours of irradiance, and never attained the highest RuBP levels observed in the control chamber plants. Measurements of canopy net photosynthetic rates between 1300 and 1500 EST indicated that, despite the higher RuBP levels of the control chamber plants, the whole-canopy photosynthetic rates were similar during this period: 28.4 and 27.5 mg CO_2/dm^2

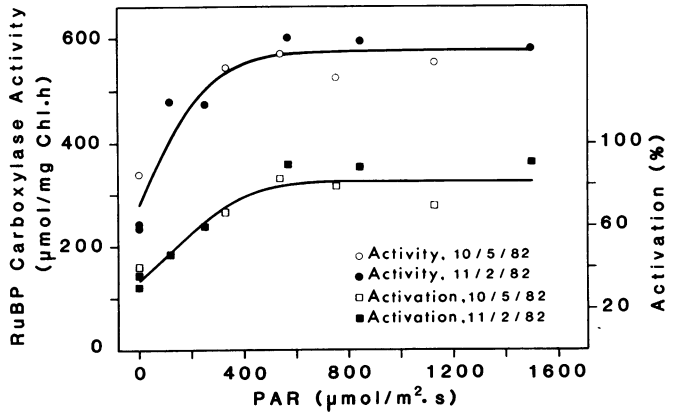

FIG. 3. HCO_3^-/Mg^{2+}-activated RuBPCase values (○, ●) and per cent RuBPCase activation (□, ■) as a function of the PAR from soybean leaves sampled on October 5, 1982, and November 2, 1982. The plants were grown at 330 μl CO_2/l.

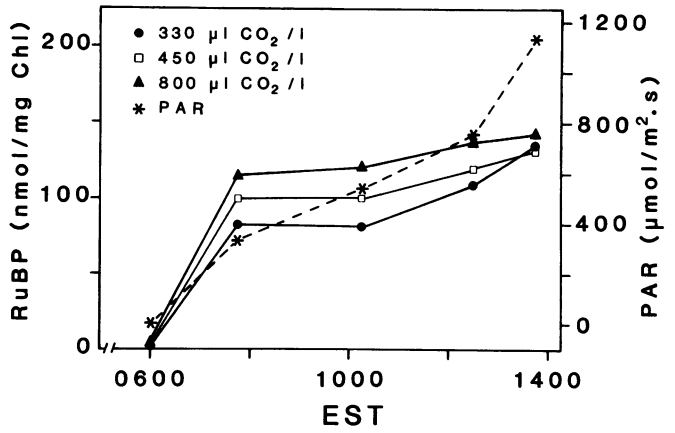

FIG. 4. Diurnal changes in the RuBP levels of leaves from 53-d-old soybean plants grown under three CO_2 concentrations (330, 450, and 800 μl CO_2/l). The leaf samples were harvested, at the time shown, on October 5, 1982. The PAR at the time of sampling is plotted for reference.

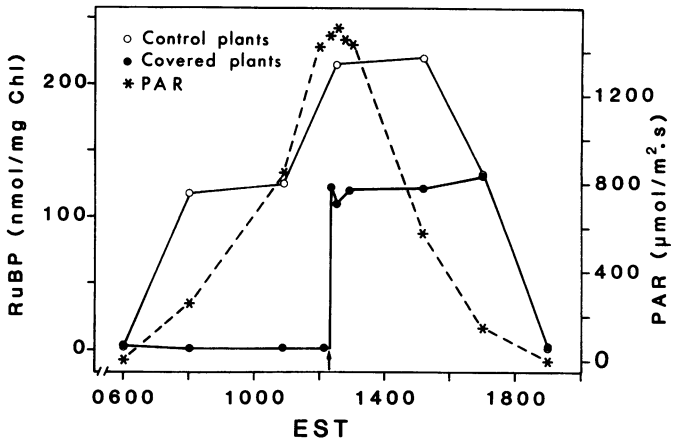

FIG. 5. Diurnal changes in the RuBP levels of leaves from 81-d-old soybean plants grown at 330 μl CO_2/l. The control plants were exposed to natural solar irradiance throughout the day. The covered plants were kept dark until noon, at which time the chamber cover was removed to expose the plants to solar irradiance (arrow). The leaf samples were harvested, at the time shown, on November 2, 1982. The PAR at the time of sampling is plotted for reference.

ground area·h for the control and the previously covered chambers, respectively (Pierce Jones, L. H. Allen, Jr., and J. W. Jones, unpublished data). Whole-canopy transpiration rates also were similar.

At 1700 EST, when the PAR was 150 μmol/m^2·s, the RuBP levels from leaves of the control and the previously covered chamber were similar (130 nmol/mg Chl). At 1900 EST, when light was no longer available, the RuBP levels in leaves of both chambers declined to near zero.

DISCUSSION

In green leaves, the amount of RuBPCase and the degree to which it is activated under given environmental conditions are important factors in the regulation of CO_2 assimilation (4, 19). Our data indicate that there were diurnal changes in RuBPCase activities in soybean leaves, with up to a 3-fold increase in activity occurring during the day over the nighttime values. This fluctuation in enzyme activity was apparently not due to an internal diel rhythm, but was cued by the environment. Thus RuBPCase extracted from plants held in darkness throughout the morning hours exhibited a low activity that was comparable to that from leaves taken before sunrise. When the cover was removed at noon, the enzyme activities doubled, after only 5 min of exposure to midday solar irradiance (1500 μmol/m^2·s). Because of the rapidity with which this large increase in enzyme activity occurred, it is unlikely to be due to *de novo* protein synthesis. It is probable that the effect of the light is to shift the enzyme into a more active catalytic form.

It is well established that in order to form the active state, the enzyme *in vitro* requires preincubation with Mg^{2+} and CO_2 prior to the addition of RuBP (15). The physiological importance of this appears to be related to the substantial increases in stromal Mg^{2+} concentrations and pH that occur upon irradiance (21, 27) and thus could be the means whereby light activates the enzyme. However, the data presented in this study indicate that the presence of HCO_3^- and Mg^{2+} at the appropriate pH cannot fully account for the total potential activity of the enzyme, since preincubation of enzyme extracts from dark-treated plants with saturating levels of Mg^{2+} and HCO_3^- could not raise the activity to that obtained from plants exposed to light. Bahr and Jensen (4), using isolated chloroplasts exposed to light or dark prior to assay of RuBPCase, concluded that part of the light activation may be due to increased Mg^{2+} in the stroma; however, the inhibition of light activation by DL-glyceraldehyde suggested that *in vivo* some PCR cycle intermediate also may be involved. A number of workers have reported that compounds such as NADPH, 6-P-gluconate, and fructose-P_2 may increase the enzyme activity *in vitro*, but usually only when preincubated with the enzyme at suboptimal Mg^{2+} and HCO_3^- concentrations (6, 7, 10, 17). These positive effectors appear to bind to the enzyme and stabilize the active ternary form containing CO_2 and Mg^{2+} (17). It should be noted that in the present study, *saturating* HCO_3^- and Mg^{2+} concentrations failed to fully activate the enzyme extracted from darkened leaves. Thus, the component (or components) involved in this light activation of soybean RuBPCase was not restricted to exerting its effect at suboptimal HCO_3^- and Mg^{2+} levels. Furthermore, whatever the cause of the light activation, apparently it was not diluted out or negated by the extraction process.

From the data in Figures 1 and 2, when the percentages of the ratios of nonactivated to HCO_3^-/Mg^{2+}-activated enzyme values were computed for each sampling time point and CO_2 treatment, they ranged from 60 to 90%. The remaining range of 40 to 10% could be attributed to the activation due to the addition of HCO_3^- and Mg^{2+}. Similarly, in Figure 3, the per cent activation curve as a function of light leveled out at about 80%; the remaining 20% should represent the HCO_3^-/Mg^{2+}-activation component. In comparison, McCurry *et al.* (17) have pointed out that only about 18 to 20% of the enzyme would be in the active ternary form at physiological HCO_3^-, Mg^{2+}, and pH levels. It appears that further investigation of this light activation phenomenon is required before the *in vivo* activation of RuBPCase can be fully explained.

The level of RuBP in soybean leaves was also regulated by sunlight. In the light, the RuBP levels ranged from about 100 to 230 nmol/mg Chl. Créach and Stewart (8) have reported 75 to 100 nmol RuBP/mg Chl in chamber-grown soybeans, and in wheat seedlings values of 250 to 300 were observed during steady state photosynthesis (19). In the present study, using soybeans grown under natural sunlight, there was some indication that a higher quantum irradiance than the 225 μmol/m^2·s reported for wheat (19) was required to induce the highest RuBP levels, because the RuBP levels measured on the overcast October sampling date never reached the maximum values observed on November 2, 1982.

As has been reported for isolated spinach chloroplasts (22) and wheat seedlings (19), RuBP levels in the dark were low, and in the case of the soybean leaves they were close to zero. They remained very low in the covered chamber throughout the morning; however, upon exposure to sunlight at noon, RuBP rose rapidly. A similar rapid rise in RuBP levels, within 1 or 2 min, has been observed in wheat (19) upon switching from dark to light. The rapid rise in soybean RuBP levels upon exposure to light paralleled the increase in RuBPCase activity. In the present study, it is not clear why the RuBP levels failed to reach those of the control leaves, even after several hours of full sun exposure. Data from the control and covered chambers on canopy photosynthetic and transpiration rates were similar, after removal of the cover. This suggests that differences in stomatal closure were not the cause of the variation in the maximum RuBP levels. To attain the highest RuBP levels in soybean leaves, it may be that both high irradiance and a relatively prolonged exposure period are required. Furthermore, from these observations it would appear that RuBP levels were not a major factor limiting soybean photosynthetic rates, at least for the greater part of the day.

The results from this study indicate that increasing atmospheric CO_2 levels during growth affect the levels of RuBP and the RuBPCase activity of soybean leaves. Increased CO_2 during growth increased the RuBP levels, but decreased RuBPCase activity especially during the earlier growth stages. Recently, CO_2 enrichment, to a doubling of atmospheric levels, has been reported to reduce RuBPCase activity in other C_3 species. These include *Atriplex triangularis*, *Nerium oleander* (9), and cotton (28). In *N. oleander*, the reduction in RuBPCase activity was associated with a reduced amount of RuBPCase protein at the higher CO_2 level (9). This reduction in RuBPCase activity, and possibly RuBPCase protein, in C_3 species at elevated atmospheric CO_2 concentrations is analogous to the lower levels of the enzyme found naturally in C_4 plants which possess an internal CO_2 concentrating mechanism (9). Although in soybean leaves, as in C_4 plants, the RuBPCase activity was somewhat depressed, this was more than compensated for by the stimulatory effect of increased CO_2 on C_3 photosynthesis. Thus, in a related study on these soybeans, net photosynthetic rate (measured at the elevated CO_2 level used for growth), starch content, plant weight, and seed yield were all found to be enhanced by CO_2 enrichment (26). Consequently, it appears that an increase in atmospheric CO_2 would increase soybean production, despite a concomitant minor reduction in RuBPCase activity.

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