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Effects of elevated CO_2 and temperature on photosynthesis and Rubisco in rice and soybean

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ABSTRACT

Rice (*Oryza sativa* L. cv. IR-72) and soybean (*Glycine max* L. Merr. cv. Bragg), which have been reported to differ in acclimation to elevated CO_2, were grown for a season in sunlight at ambient and twice-ambient [CO_2], and under daytime temperature regimes ranging from 28 to 40 °C. The objectives of the study were to test whether CO_2 enrichment could compensate for adverse effects of high growth temperatures on photosynthesis, and whether these two C_3 species differed in this regard. Leaf photosynthetic assimilation rates (A) of both species, when measured at the growth [CO_2], were increased by CO_2 enrichment, but decreased by supraoptimal temperatures. However, CO_2 enrichment more than compensated for the temperature-induced decline in A. For soybean, this CO_2 enhancement of A increased in a linear manner by 32–95% with increasing growth temperatures from 28 to 40 °C, whereas with rice the degree of enhancement was relatively constant at about 60%, from 32 to 38 °C. Both elevated CO_2 and temperature exerted coarse control on the Rubisco protein content, but the two species differed in the degree of responsiveness. CO_2 enrichment and high growth temperatures reduced the Rubisco content of rice by 22 and 23%, respectively, but only by 8 and 17% for soybean. The maximum degree of Rubisco down-regulation appeared to be limited, as in rice the substantial individual effects of these two variables, when combined, were less than additive. Fine control of Rubisco activation was also influenced by both elevated [CO_2] and temperature. In rice, total activity and activation were reduced, but in soybean only activation was lowered. The apparent catalytic turnover rate (K_{cat}) of rice Rubisco was unaffected by these variables, but in soybean elevated [CO_2] and temperature increased the apparent K_{cat} by 8 and 22%, respectively. Post-sunset declines in Rubisco activities were accelerated by elevated [CO_2] in rice, but by high temperature in soybean, suggesting that [CO_2] and growth temperature influenced the metabolism of 2-carboxyarabinitol-1-phosphate, and that the effects might be species-specific. The greater capacity of soybean for CO_2 enhancement of A at supraoptimal temperatures was probably not due to changes in stomatal conductance, but may be partially attributed to less down-regulation of Rubisco by elevated [CO_2] in soybean than in rice. However, unidentified species differences in the temperature optimum for photosynthesis also appeared to be important. The responses of photosynthesis and Rubisco in rice and soybean suggest that among C_3 plants species-specific differences will be encountered as a result of future increases in global [CO_2] and air temperatures.

Key-words: *Oryza sativa*; *Glycine max*; global climate change; Rubisco regulation.

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INTRODUCTION

The global atmospheric CO_2 concentration, presently about 360 μmol mol^{-1}, is increasing and is expected to double by the end of the next century (King *et al.* 1992). Atmospheric general circulation models predict that the increase in CO_2 and other 'greenhouse' gases may cause global air temperatures to rise, possibly by as much as 3–6 °C (Wilson *et al.* 1987; Hansen *et al.* 1988).

The present atmospheric [CO_2] is an important limiting factor for the photosynthesis, growth and productivity of many crop species. In a leaf, the photosynthetic rate is a direct result of the activity of ribulose bisphosphate carboxylase-oxygenase (Rubisco), which in turn is influenced by various environmental factors, including CO_2, temperature, and light. The current atmospheric [CO_2] is insufficient to saturate Rubisco in C_3 plants. Consequently, in short-term measurements an increase in the availability of this substrate results in a rise in leaf photosynthetic rates, partly because high [CO_2] inhibits the oxygenase reaction of Rubisco and the subsequent loss of CO_2 through photorespiration (Bowes 1993). However, for some species, longer exposure to elevated [CO_2] results in acclimation of photosynthesis with down-regulation of the amount of Rubisco protein (Rowland-Bamford *et al.* 1991), although other species show minimal down-regulation (Campbell *et al.* 1988; Sage *et al.* 1989; Socias *et al.* 1993). This coarse control of the amount of Rubisco protein probably serves to optimize CO_2 acquisition with utilization of the fixed carbon (Woodrow 1994).

In addition to coarse control of Rubisco protein, there are fine controls which respond more rapidly to changes in

environmental conditions. In this regard, two major mechanisms are known to regulate Rubisco activity. One involves the reversible carbamylation of a lysine residue in the active site to activate the enzyme, while the other operates by the reversible binding of 2-carboxyarabinitol-1-phosphate (CA1P), the Rubisco dark inhibitor, to the carbamylated site (Vu *et al.* 1983; Seemann *et al.* 1990; Portis 1992; Sage & Reid 1994). The carbamylation of Rubisco is dependent upon Rubisco activase, which catalytically removes RuBP, CA1P and other inhibitory phosphorylated compounds from the catalytic sites (Salvucci 1989; Seemann *et al.* 1990; Servaites 1990; Portis 1992). Rubisco activation responds to relatively rapid changes in irradiance and CO_2 or O_2, while the regulation by CA1P seems to occur only in response to irradiance, predominantly low light and most obviously darkness (Salvucci 1989; Sage & Reid 1994). There are a number of reports which show that long-term growth at elevated [CO_2] can cause a reduction in Rubisco activation, but it is unclear whether rising [CO_2] also influences the fine control of Rubisco exercised by CA1P (Bowes 1993).

In addition to CO_2, the photosynthetic rates of C_3 plants are affected by temperature, and this effect is also primarily exerted through Rubisco (Long 1991). An increase in temperature reduces the activation state of the enzyme (Kobza & Edwards 1987; Holaday *et al.* 1992), and decreases both the specificity for CO_2 and the solubility of CO_2, relative to O_2 (Jordan & Ogren 1984; Brooks & Farquhar 1985; Long 1991). The latter two effects result in greater losses of CO_2 to photorespiration as the temperature rises. Consequently, a doubling of atmospheric [CO_2], and the concomitant inhibition of the Rubisco oxygenase reaction, should moderate the adverse effects of high temperature on C_3 photosynthesis, and result in even greater enhancement of net photosynthesis by elevated [CO_2] as growth temperatures increase (Long 1991). However, the data in this regard are equivocal (Farrar & Williams 1991), and there is little information as to whether temperature itself impacts the coarse control of Rubisco protein, or fine control of activity via CA1P. Furthermore, the degree to which elevated CO_2 or temperature causes down-regulation of Rubisco should influence the amount of enhancement by elevated [CO_2].

In this study, two C_3 crop species, rice, which shows marked acclimation to elevated [CO_2] (Rowland-Bamford *et al.* 1991), and soybean, which appears less affected (Campbell *et al.* 1988), were grown for a season in ambient- or enriched-CO_2 atmospheres with various temperature regimes. One objective was to test the hypothesis that the enhancement effect of elevated [CO_2] on leaf photosynthetic rates increases with temperature, and to determine if it could compensate for adverse effects of high temperatures, even after long-term acclimation to the growth regimes. A further objective was to ascertain whether temperature and CO_2 exhibit interactive effects in exerting coarse and/or fine control over Rubisco activity, and whether there were species differences in this regard.

MATERIALS AND METHODS

Plant material and growth conditions

Rice (*Oryza sativa* L. cv. IR-72) and soybean (*Glycine max* L. Merr. cv. Bragg) were grown for a season in eight sunlit, controlled-environment growth chambers (also known as Soil-Plant-Atmosphere-Research, or SPAR, units) located outdoors in Gainesville, Florida. The above-ground chambers, 2 m × 1 m in cross-section and 1·5 m high, were covered with clear polyester Mylar so that plants received direct, natural solar irradiance. The chamber tops were attached to aluminum vats (2 m × 1 m in area and 0·6 m deep), which provided water-tight, rooting environments for rice growth. Each vat was filled with soil to a depth of 0·5 m and, prior to planting, the soil in each chamber was fertilized with P and K at a rate of 9·0 g m^{-2}. Rice was planted on 20 July 1992, and 8 d later the vats were flooded with water which was maintained at 5 cm above the soil surface with the aid of a float-actuated water valve. Nitrogen was applied as urea at a rate of 12·6, 6·3 and 6·3 g m^{-2} at 7, 31 and 63 d after planting. The rice plants were grown throughout their life cycle at two daytime CO_2 concentrations: 330 and 660 μmol mol^{-1}. The dry bulb air temperatures followed a sinusoidal diurnal pattern and were controlled at day/night maximum/minimum values of 32/23, 35/26 and 38/29 °C for each CO_2 treatment, and dewpoint temperatures were maintained constantly at 18, 21 or 24 °C, respectively, for the three air temperatures (Baker *et al.* 1994).

Soybean seeds were first inoculated with *Rhizobium* and were then planted on 19 August 1993 at a row spacing of 33 cm, resulting in a total of 40 plants m^{-2} at mid-growth season. Plastic drainage pipes 4 cm in diameter were laid at the bottom of each chamber, and were covered with gravel to the pipe thickness and then by a 2 cm coarse-sand layer. A fine-sand soil profile was then constructed above the sand layer. These soil bins (2 m × 1 m in area and 0·6 m deep) therefore provided a large soil volume for soybean plant roots in the growth chambers. After the first 10 d, plants were watered continuously by sub-irrigation. The soybean plants were grown throughout their life cycle at two daytime CO_2 concentrations of 350 and 700 μmol mol^{-1}. The dry bulb day/night maximum/minimum air temperatures were controlled at 28/18, 32/22, 36/26 and 40/30 °C for each CO_2 treatment, and day/night dewpoint temperatures were maintained at 12/10, 16/12, 20/14 and 24/16 °C, respectively, for the four air temperature treatments. Both the dry bulb air and dewpoint temperatures followed a sinusoidal control set point that varied continuously between maximum (daytime) and minimum (nighttime) values. Jones *et al.* (1984), Baker *et al.* (1994) and Pickering *et al.* (1994) describe the detailed chamber characteristics, specific methods for controlling chamber environmental set points, and the quality of these environmental controls, with daily print-outs of diurnal trends of the control variables, including desired set-point CO_2/air temperatures and measured CO_2/air temperatures, to ensure the identical performance of the chambers throughout the plant growth season.

Photosynthesis measurements

Photosynthesis of single, attached, fully expanded sun leaves was measured with a LI-COR LI-6200 Portable Photosynthesis System in a closed mode, at 90 d after planting for rice and 60 d after planting for soybean. Leaf photosynthetic measurements were performed at midday, between 1000 and 1300 eastern day time (EDT), when the solar photosynthetic photon irradiance was saturating at 1200–1600 μmol m^{-2} s^{-1}. The LI-COR LI 6000-13 0·25 dm^3 cuvette was used for rice, and the LI-COR LI 6000-12 1 dm^3 cuvette for soybean. The duration of each measurement was typically 30–45 s. Photosynthetic rates were expressed on a leaf area basis.

Leaf sampling

Diel samplings of leaves for each CO_2 and growth temperature treatment were performed, 60 d after planting for rice and 53 d for soybean, starting 1 h before sunrise and ending at least 1 h after sunset. A total of three samplings were taken during the day for each treatment. At each sampling time, 10 uppermost fully expanded leaves were detached from 10 different plants for each treatment, immediately immersed in liquid N_2, pooled together, ground to a fine powder in liquid N_2 with a mortar and pestle, and then stored in liquid N_2 until analyses for Rubisco activity, Rubisco protein content and RuBP.

Extraction and assay of Rubisco

A portion of the frozen leaf powder, about 150 mg, was transferred to a pre-chilled Ten Broeck homogenizer and ground at 2 °C in 3·5 cm^3 of extraction medium which consisted of 50 mol m^{-3} Bicine-NaOH, 10 mol m^{-3} $MgCl_2$, 5 mol m^{-3} DTT, 10 mol m^{-3} D-isoascorbate, 0·1 mol m^{-3} EDTA-Na_2, and 2% (w/v) PVP-40 at pH 8·0. The presence of 0·1% (v/v) Triton X-100 did not increase the extractable activity, and this was not included. Similarly, activity was not increased by treatment of crude extracts with 200 mol m^{-3} Na_2SO_4 for 30 min at 2 °C followed by desalting on G-25 Sephadex columns. The homogenate was micro-centrifuged at 12 000 *g* for 45 s at 2 °C, and an aliquot of the supernatant was immediately assayed for Rubisco activity. This procedure from extraction to assay took ≈4 min, and the initial activity was measured to reflect the *in vivo* activity of Rubisco. Assay reactions were performed at 30 °C in a total volume of 0·5 cm^3. The reaction mixture consisted of 50 mol m^{-3} Tris-HCl, 5 mol m^{-3} DTT, 10 mol m^{-3} $MgCl_2$, 0·1 mol m^{-3} EDTA, 0·5 mol m^{-3} RuBP and 20 mol m^{-3} $NaH^{14}CO_3$ (2·0 GBq $mmol^{-1}$) at pH 8·0. The initial Rubisco activity was measured by injecting 0·1 cm^3 of the supernatant into the assay mixture and terminating the reaction after 45 s with 0·1 cm^3 of 6 kmol m^{-3} HCl.

For measurement of the total activity that reflects the maximum activatable activity of Rubisco, a second aliquot (0·1 cm^3) of the supernatant was incubated in the reaction mixture described above, except that RuBP was omitted. After a 5 min activation period, the reaction was initiated by the addition of RuBP. The reaction was stopped after 45 s by the addition of 0·1 cm^3 of 6 kmol m^{-3} HCl. In the control assays, RuBP was omitted to ensure no other carboxylase was active in the crude extract. After assay, the mixtures were dried at 60 °C and the acid-stable ^{14}C radioactivity was determined by liquid scintillation spectrometry.

Activation of Rubisco was computed as the ratio of the initial to the corresponding total activity of daylight-sampled leaves.

Quantification of Rubisco protein

Rubisco contents in leaf tissues were determined by a modification of the radioimmuno-precipitation procedures previously reported (Collatz *et al.* 1979; Vu & Yelenosky 1988). About 100 mg of liquid N_2-frozen leaf powder was ground in 2·5 cm^3 of 50 mol m^{-3} Bicine-NaOH buffer containing 5 mol m^{-3} DTT, 0·1 mol m^{-3} EDTA, 10 mol m^{-3} $MgCl_2$, 10 mol m^{-3} $NaHCO_3$ and 2% (w/v) PVP-40 at pH 8·0. The homogenate was microcentrifuged at 12 000 *g* for 1 min at 2 °C. A 25 mm^3 aliquot of the supernatant was then added to 50 mm^3 of buffer (100 mol m^{-3} Bicine, 20 mol m^{-3} $MgCl_2$, 1 mol m^{-3} EDTA at pH 7·8) containing 4 nmol [2–^{14}C] CABP and 50 mm^3 of antiserum to purified tobacco Rubisco raised from rabbits. These concentrations of supernatant and antiserum were established through titration experiments to ensure complete precipitation of the rice and soybean Rubisco protein. After incubation for 2 h at 37 °C, the precipitate was collected on a Millipore cellulose acetate/nitrate filter (0·45 μm pore size) and washed with 5 cm^3 of a 0·85% (w/v) NaCl solution containing 10 mol m^{-3} $MgCl_2$, and the bound ^{14}C was determined by liquid scintillation counting.

The apparent catalytic turnover rates (K_{cat}) of fully activated Rubisco from rice and soybean leaves harvested during the middle part of the day were computed based on the midday total Rubisco activities, the Rubisco protein contents, and a molecular weight of 550 000 for the Rubisco protein.

Extraction and determination of RuBP

RuBP was extracted from the liquid N_2-frozen leaf powder with 0·5 kmol m^{-3} HCl. After centrifugation at 12 000 *g* for 5 min at 2 °C, the supernatant was adjusted to pH 8·3 with 2 kmol m^{-3} Tris-Base and 4 kmol m^{-3} KOH. RuBP was then assayed using purified tobacco Rubisco as described by Vu *et al.* (1983).

Each value of leaf photosynthetic rates represents the mean and standard error from measurements of six to eight plants. Data on Rubisco and RuBP are presented as the mean and standard error of three replicates.

RESULTS

Leaf photosynthetic CO_2 assimilation rates of both rice and soybean plants, when measured at the [CO_2] used for growth, were substantially enhanced by elevated CO_2, even after 2–3 months of growth at high [CO_2] (Fig. 1).

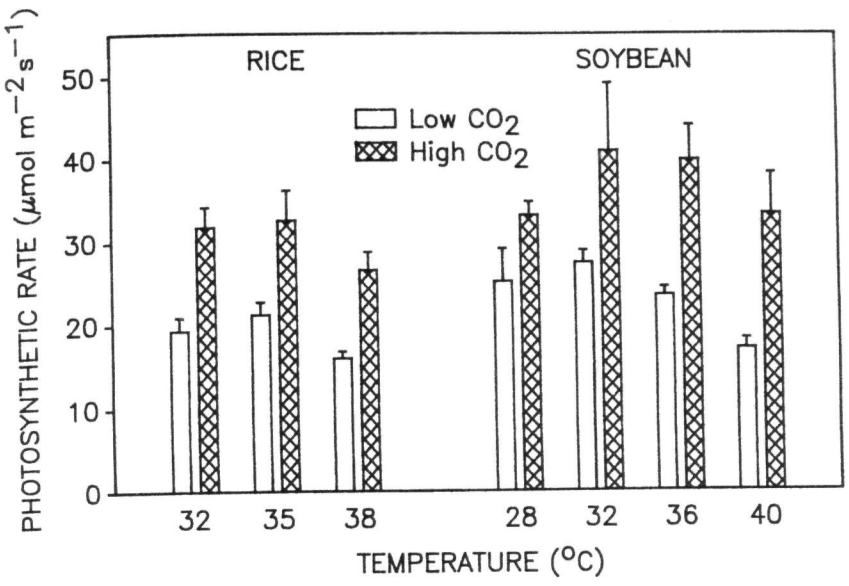

Figure 1. Midday leaf photosynthetic rates of rice and soybean grown under two CO_2 levels and various temperature regimes. The temperatures shown are the daytime air temperatures. Midday solar photosynthetic photon irradiance during measurements, from 1000 to 1300 EDT, was 1200–1600 μmol m^{-2} s^{-1}.

Values for rice ranged from 16·0 to 21·2 and from 26·6 to 32·6 μmol m^{-2} leaf area s^{-1} at ambient and elevated CO_2, respectively. The corresponding rates for soybean ranged from 17·1 to 27·5 and from 33·2 to 40·9 μmol CO_2 m^{-2} leaf area s^{-1}. Thus, the photosynthetic rate was enhanced by elevated [CO_2] at all growth temperatures used. Under both [CO_2] regimes, net photosynthetic rates were highest at 35 and 32 °C for rice and soybean, respectively, but declined with higher or lower growth temperatures.

For rice, the percentage enhancement in photosynthetic rate due to a doubling in [CO_2] was not markedly altered over the range of growth temperatures used (Fig. 2). Soybean, in contrast, showed a linear increase in the CO_2 enhancement of photosynthesis with increasing growth temperature, such that the degree of enhancement rose from 32% at 28 °C to 95% at 40 °C (Fig. 2).

Determination of the ratio of intercellular CO_2 concentration (C_i) and atmospheric CO_2 concentration (C_a) as a function of growth conditions indicated that it was not changed by increasing temperature or [CO_2], and only a small difference was found between the two species. For rice the mean C_i/C_a ratios as functions of temperature and [CO_2] were 0·93 ± 0·01 and 0·94 ± 0·02, respectively. Comparable values for soybean were 0·87 ± 0·02 and 0·83 ± 0·03, respectively.

Diel activities of Rubisco from rice plants grown at the three temperature and two [CO_2] regimes are shown in Table 1. Both increasing growth temperature and [CO_2] reduced the initial and total Rubisco activities assayed at 30 °C of leaves sampled in the light (1000 EDT). Thus, at ambient [CO_2], an increase in growth temperature from 32 to 38 °C reduced the total Rubisco activity by 21·5%, while at 32 °C a doubling of [CO_2] reduced it by 17·7%. However, these down-regulatory effects of temperature and [CO_2] were not additive, as at the highest growth temperature and [CO_2] total Rubisco activity was down-regulated only by 27·5%, not by a cumulative 39·2% (i.e. 21·5% + 17·7%). Because initial activity was down-regulated more than total activity, Rubisco activation in daylight-sampled leaves was also reduced by both growth treatments, with values declining from 90·5% at 32 °C and 330 μmol CO_2 mol^{-1} to only 68·4% at 38 °C and 660 μmol CO_2 mol^{-1} (Table 1). In this case the combined effects of temperature and [CO_2] did appear to be additive.

In terms of light/dark regulation, the initial and total Rubisco activities of rice in all growth treatments were substantially lower before sunrise, and increased 3- to 5-fold as daylight increased (Table 1). Pre-dawn activities did not differ greatly among the temperature regimes, but were as much as 27% lower in elevated [CO_2]. After sunset, the initial activity of the elevated [CO_2] plants declined more rapidly than that of plants at ambient [CO_2], irrespective of the temperature treatment. Total activity also declined after sunset, but at a rate considerably slower than the initial activity, and this phenomenon was more evident with the ambient [CO_2] treatment.

The corresponding diel Rubisco activities for soybean are shown in Table 2. Unlike rice, total activities for soybean leaves sampled in the light (1130 EDT) were hardly affected by elevated [CO_2] (1·1–6·6% less), and not at all by high temperature. However, both elevated temperature and [CO_2] growth regimes resulted in lower initial Rubisco activities. Consequently, Rubisco activation in the light was reduced by increasing growth temperature and [CO_2] (Table 2), but the combined effect of these two variables was not additive.

For both rice and soybean, extractable activity was treated with Na_2SO_4 to determine whether total activity could be increased (Parry *et al.* 1994). This treatment did not enhance activity in either species (data not shown), which indicated that total and maximal activities were equivalent for these plants.

The light/dark regulation of soybean Rubisco was also affected by the growth regime. Initial and total activities were low before sunrise and increased 1·2- to 5·5-fold by 1130 EDT, but pre-dawn values for both activities were reduced by growth at elevated temperature or [CO_2]. At the lowest growth temperature, pre-dawn total activities were on average 75% of the corresponding midday value,

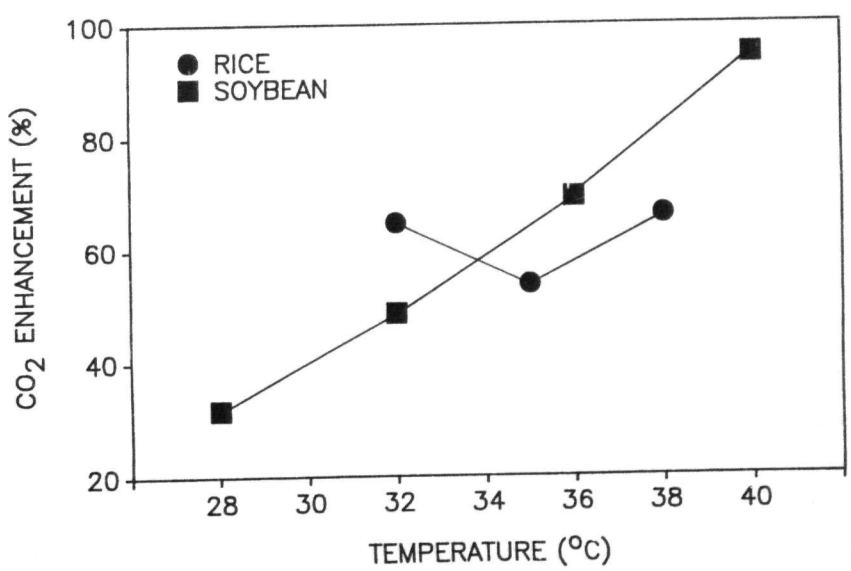

Figure 2. Per cent elevated-CO_2 enhancement in leaf photosynthetic rates of rice and soybean over the range of growth temperatures.

Growth conditions		Time of day (EDT)	Rubisco activity*		Activation (%)
Temperature (°C)	[CO_2] (μmol mol^{-1})		Initial (μmol g^{-1} leaf FW h^{-1})	Total	
32/22	330	0600	251 (7)	383 (4)	18·0
		1000	1266 (19)	1399 (29)	90·5
		2000	473 (12)	1117 (43)	33·8
	660	0600	231 (9)	339 (5)	20·0
		1000	917 (49)	1151 (12)	79·7
		2000	348 (4)	740 (24)	30·2
35/26	330	0600	245 (5)	381 (4)	16·8
		1000	1271 (28)	1462 (32)	86·9
		2000	497 (9)	1180 (14)	34·0
	660	0600	194 (6)	279 (7)	17·4
		1000	954 (43)	1117 (55)	85·4
		2000	293 (5)	586 (4)	26·2
38/29	330	0600	245 (8)	373 (6)	22·3
		1000	868 (30)	1098 (27)	79·0
		2000	369 (10)	1024 (4)	33·6
	660	0600	205 (4)	322 (8)	20·2
		1000	693 (10)	1014 (14)	68·4
		2000	279 (5)	600 (4)	27·6

*Values are the mean and standard error (parentheses) of three determinations.

Table 1. Initial and total activities and activation of Rubisco extracted from leaves of rice plants grown under two CO_2 levels and three temperature (day/night) regimes. Solar photon irradiances at 0600, 1000 and 2000 EDT leaf samplings were 0, 950 and 0 μmol m^{-2} s^{-1}, respectively

whereas this value was only 26%, for plants grown at the highest temperature. Similarly, after sunset, total Rubisco activities remained substantially greater for plants at the lower growth temperatures. Elevated [CO_2] also caused lower pre-dawn and post-sunset initial and total Rubisco activities, though the reductions were much less than for the high temperature regimes. In addition to activity, leaves sampled in the dark, either before dawn or after sunset, exhibited lower Rubisco activation when taken from plants at elevated [CO_2], and the lowest activation values occurred with plants grown at the highest temperature (Table 2).

Treatments of the crude extracts from dark-sampled rice and soybean leaves with saturated $(NH_4)_2SO_4$ solution to yield a 30–60% fractionation precipitate entirely removed the inhibitory effect of the dark (data not shown).

Table 3 shows the Rubisco protein content of rice and soybean leaves under the differing temperature and [CO_2] treatments. For rice, CO_2 enrichment reduced the Rubisco content by 20·7% at 32 °C, which is equivalent to the reduction found for total Rubisco activity (Table 1), whereas for soybean at the same growth temperature the reduction was only 8·4%. Increasing temperatures also reduced the Rubisco protein content (Table 3). In the case of rice at ambient [CO_2], an increase in growth temperature from 32 to 38 °C resulted in a 22·6% reduction in Rubisco protein, while for soybean increasing the temperature from 32 to 40 °C produced a 17·4% reduction. Thus, for soybean, the temperature regime had more effect on Rubisco protein content than [CO_2], whereas for rice, both environmental factors exerted coarse control effects on Rubisco.

Rice and soybean differed in the response of the Rubisco apparent catalytic turnover rate (K_{cat}) to the growth conditions (Table 3). In rice, the apparent K_{cat} of fully activated Rubisco in the light was unaffected by either the temperature or [CO_2] used for growth, wheareas in soybean the apparent K_{cat} of fully activated Rubisco increased by about 21·5% with increasing growth temperature. There was also a small (5–8%) increase in apparent K_{cat} in response to elevated [CO_2]. This up-regulation effect of temperature and [CO_2] was additive. The increase in apparent K_{cat} was not a function of assay temperature, as this was held constant at 30 °C.

Table 4 depicts the diel RuBP contents in rice and soybean leaves under the various growth treatments. For rice, the leaf RuBP content before dawn was in the range of 3–4 nmol g^{-1} leaf fresh weight but rose over 100-fold by mid-morning. Except at the highest temperature, the mid-morning RuBP values were lower in the CO_2-enriched plants. After sunset, RuBP showed a substantial decline, but the content in the ambient-CO_2 plants was consistently about 2-fold higher than that in the enriched plants, irrespective of temperature. For soybean leaves, the pre-dawn values were lower than in rice, but they also increased, by 100- to 200-fold, as the day progressed. Unlike rice, no obvious trends were discernible as a function of the growth conditions, and they dropped into the range of the pre-dawn values after sunset. Based on the values for Rubisco protein content (Table 3), a molecular weight of 550 000 for the Rubisco protein, and eight binding sites for RuBP, the midday RuBP pools resulted in an average of 2 mol RuBP mol^{-1} binding site of Rubisco for rice, compared to 1 mol RuBP mol^{-1} binding site of Rubisco for soybean.

Table 2. Initial and total activities and activation of Rubisco extracted from leaves of soybean plants grown under two CO_2 levels and four temperature (day/night) regimes. Solar photon irradiances at 0600, 1130 and 2000 EDT leaf samplings were 0, 1600 and 0 μmol m^{-2} s^{-1}, respectively

Growth conditions		Time of day (EDT)	Rubisco activity*		Activation (%)
Temperature (°C)	[CO_2] (μmol mol^{-1})		Initial (μmol g^{-1} leaf FW h^{-1})	Total	
28/12	350	0630	749 (2)	1284 (11)	48·0
		1130	1477 (24)	1561 (14)	94·6
		2000	813 (30)	1386 (13)	52·1
	700	0630	568 (9)	1039 (15)	37·5
		1130	1231 (19)	1516 (9)	81·1
		2000	720 (25)	1104 (11)	47·5
32/23	350	0630	575 (8)	1062 (17)	36·3
		1130	1537 (12)	1583 (29)	97·2
		2000	772 (12)	1333 (11)	48·8
	700	0630	458 (2)	794 (16)	29·2
		1130	1307 (37)	1566 (18)	83·4
		2000	596 (15)	1079 (46)	38·1
36/26	350	0630	311 (2)	539 (6)	19·5
		1130	1362 (12)	1593 (15)	85·5
		2000	538 (15)	955 (25)	33·8
	700	0630	262 (6)	467 (3)	16·8
		1130	1218 (13)	1556 (23)	78·3
		2000	354 (9)	709 (7)	22·8
40/30	350	0630	225 (3)	418 (5)	14·1
		1130	1188 (17)	1596 (21)	74·4
		2000	361 (6)	866 (21)	22·6
	700	0630	193 (7)	382 (5)	13·0
		1130	1063 (3)	1490 (8)	71·4
		2000	308 (4)	666 (8)	20·7

*Values are the mean and standard error (parentheses) of three determinations.

DISCUSSION

A comparison of net photosynthetic rates for the optimum and highest growth temperature regimes showed that at ambient [CO_2] the high temperature treatments reduced the rice and soybean leaf rates by 25 and 38%, respectively. Despite this potentially deleterious temperature effect, for both species the CO_2-enriched plants in the high temperature regimes outperformed their ambient-CO_2 counterparts growing at optimum temperatures. Thus the elevated [CO_2] more than compensated for the adverse effects of high temperatures on net photosynthesis. However, the CO_2 enhancement response of rice, as a function of temperature, differed somewhat from that of soybean. In rice, a doubling of the growth [CO_2] resulted in 55–65% enhancement of net photosynthesis over the 6 °C range used, whereas in soybean the degree of enhancement rose linearly, to a value of 95%, with increasing temperature from 28 to 40 °C. The fact that the C_i/C_a ratios were not altered by elevated temperature or [CO_2] suggests that it is unlikely that the differences between soybean and rice in the degree of enhancement by CO_2 enrichment with increasing temperature can be attributed to any differential effects of temperature on stomatal conductance.

Predictions based on Rubisco kinetics indicate that, for short-term measurements of light-saturated photosynthetic rates, the degree of enhancement by elevated CO_2 should increase at higher leaf temperatures. This general scenario was observed for soybean. However, calculations for an idealized C_3 plant indicate a rise in temperature from 28 to 40 °C should increase the degree of enhancement from about 66 to 190% when the [CO_2] is raised from 350 to 650 μmol mol^{-1} (Long 1991; Fig. 2). This is substantially greater than the 32–95% enhancement found with soybean when the [CO_2] was raised to 700 μmol mol^{-1} over the same temperature range. The difference may be partially attributed to the fact that in the present study the temperature optimum of 32 °C for soybean under ambient CO_2 was 7 °C greater than that of the model C_3 plant. The shift to a higher optimum reduced the adverse temperature effects, and concomitantly the potential for CO_2 enhancement, even at 40 °C.

A somewhat similar explanation can be posited for rice, which over the 32–38 °C temperature range showed on average only about 60% CO_2 enhancement, as compared with a calculated 80–160% (Long 1991). A 3 °C change either side of the 35 °C optimum for rice appears to be too small to produce a major difference in photosynthetic enhancement when the temperature optimum under ambient [CO_2] is high and relatively broad. These data suggest that among C_3 species differences in the temperature opti-

Table 3. Contents and apparent catalytic turnover rates (K_{cat}) of Rubisco in leaves of rice and soybean grown under two CO_2 levels and various temperature (day/night) regimes

Plant	Growth conditions: Temperature (°C)	Growth conditions: $[CO_2]$ (μmol mol^{-1})	Rubisco content* (mg g^{-1} leaf FW)	Apparent K_{cat}* (mol CO_2 mol^{-1} Rubisco s^{-1})
Rice				
	32/23	330	16·4 (0·8)	13·0 (0·4)
		660	13·0 (0·7)	13·6 (0·2)
	35/26	330	15·7 (1·1)	14·2 (0·4)
		660	12·3 (0·9)	13·9 (1·0)
	38/29	330	12·7 (0·9)	13·2 (0·5)
		660	11·5 (0·5)	13·5 (0·3)
Soybean				
	28/18	350	15·3 (0·8)	15·5 (0·2)
		700	13·8 (0·2)	16·8 (0·1)
	32/22	350	15·5 (0·2)	15·6 (0·4)
		700	14·2 (0·3)	16·8 (0·3)
	36/26	350	14·5 (0·3)	16·8 (0·2)
		700	13·1 (0·6)	18·1 (0·4)
	40/30	350	12·8 (0·7)	19·1 (0·4)
		700	11·3 (1·0)	20·1 (0·2)

*Values are the mean and standard error (parentheses) of three determinations.

Table 4. Contents of RuBP in leaves of rice and soybean grown under two CO_2 levels and various temperature (day/night) regimes. Solar photon irradiances at pre-dawn, daytime and post-sunset for rice leaf samplings were 0 (0600 EDT), 950 (1000 EDT) and 0 (2000 EDT) μmol m^{-2} s^{-1}, and for soybean leaf samplings were 0 (0630 EDT), 1600 (1130 EDT) and 0 (2000 EDT) μmol m^{-2} s^{-1}, respectively

Plant	Growth conditions: Temperature (°C)	Growth conditions: $[CO_2]$ (μmol mol^{-1})	RuBP content* (nmol g^{-1} leaf FW): Pre-dawn	Daytime	Post-sunset
Rice					
	32/23	330	4·1 (0·7)	499·1 (12·9)	25·5 (0·7)
		660	3·1 (0·3)	394·3 (1·8)	12·8 (0·8)
	35/26	330	3·5 (0·1)	525·9 (4·7)	22·1 (0·2)
		660	2·8 (0·6)	363·3 (2·2)	12·6 (0·5)
	38/29	330	4·1 (0·3)	412·1 (5·7)	31·0 (4·2)
		660	3·8 (0·9)	418·2 (3·4)	15·7 (3·2)
Soybean					
	28/18	350	0·3 (0·1)	158·3 (3·4)	1·7 (0·3)
		700	0·7 (0·2)	185·1 (1·2)	2·9 (0·1)
	32/22	350	0·6 (0·1)	216·4 (7·8)	1·7 (0·1)
		700	0·3 (0·1)	207·7 (2·3)	1·2 (0·1)
	36/26	350	1·4 (0·3)	164·0 (1·2)	1·5 (0·3)
		700	0·2 (0·1)	212·9 (4·1)	1·1 (0·2)
	40/30	350	0·6 (0·1)	251·3 (1·3)	1·6 (0·4)
		700	0·4 (0·1)	175·0 (3·9)	1·2 (0·1)

*Values are the mean and standard error (parentheses) of three determinations.

mum for photosynthesis and growth could strongly influence the degree to which CO_2 enrichment enhances the photosynthetic rate at any given temperature. This may explain some of the literature reports of species variation in CO_2 enrichment response as a function of temperature (reviewed by Farrar & Williams 1991).

Down-regulation of Rubisco was probably also a factor in the lower than expected CO_2 enhancement at high temperatures. Decreases in Rubisco activity by varying amounts, as a result of long-term growth in elevated CO_2, have been reported for a number of C_3 species (Vu *et al.* 1983; Spencer & Bowes 1986; Campbell *et al.* 1988; Sage *et al.* 1989;

Yelle *et al.* 1989; Besford *et al.* 1990; Rowland-Bamford *et al.* 1991; Socias *et al.* 1993; Tissue *et al.* 1993). Both coarse control, by a lowering of protein content, and fine control, through decreased enzyme activation, play a role in the down-regulation of Rubisco mediated by elevated [CO_2]. Recent evidence suggests that the mechanism whereby elevated [CO_2] exerts its effect is alteration of the expression of genes encoding Rubisco at the transcriptional and/or post-transcriptional level, and that changes in pool sizes of glucose or sucrose provide the signal (Van Oosten & Besford 1994; Webber *et al.* 1994). However, only a weak correlation between increased carbohydrate pool sizes and decreased transcriptional activity was found in a field study of CO_2-enriched wheat (Nie *et al.* 1995). Supraoptimal temperatures also appear to reduce Rubisco activity, though the data are more meagre and the mechanisms less certain (Vierling & Key 1985; Holaday *et al.* 1992).

In this study, both elevated temperature and [CO_2] exerted coarse control over the Rubisco proteins of rice and soybean, but the two species differed in their degree of responsiveness. At optimum temperatures, doubling the growth [CO_2] reduced the Rubisco protein content by 22% for rice, but only by 8% for soybean. Previous studies have indicated that the Rubisco protein content of rice leaves is much more susceptible to down-regulation by elevated CO_2 than that of soybean (Campbell *et al.* 1988; Rowland-Bamford *et al.* 1991). Elevated growth temperatures (daytime) above 32 °C also reduced the Rubisco protein content; for each 1 °C rise, the Rubisco content declined by 3·8 and 2·2%, respectively, in rice and soybean leaves at ambient [CO_2]. Thus, again, soybean leaf Rubisco content was less susceptible to down-regulation than that of rice. The data for treatments where elevated temperature and [CO_2] could interact suggest that the maximum degree of down-regulation was subject to limitation. In the case of rice, both elevated temperature and [CO_2] had substantial individual effects, but their combined effect was less than additive. In contrast, the combined effect was additive in soybean, where individually both factors produced a relatively small response.

For rice, the percentage declines in Rubisco protein content due to elevated temperatures and [CO_2] were equivalent to the percentage declines in total Rubisco activities from daytime-sampled leaves. This correlation did not hold for soybean. Elevated temperatures caused no measurable decrease in daytime total Rubisco activity, and at the lower temperature regimes CO_2 enrichment caused only a 1–3% decline. This unexpected result, that soybean Rubisco protein, but not total activity, was down-regulated, indicated that the apparent K_{cat} was up-regulated by 30% over the range of growth temperatures and [CO_2] used. As observed here for rice, Sage *et al.* (1989) found no change in the Rubisco K_{cat} values of five C_3 species grown at elevated [CO_2]. The present study shows that growth temperature seems to be a more important factor in this phenomenon than [CO_2]. It is unclear whether the up-regulation of soybean apparent K_{cat} has physiological significance in maintaining the photosynthetic rate, because it was accompanied by a decline in the initial activity, and thus activation, of Rubisco. In fact, rice and soybean showed similar declines in Rubisco activation, which were caused by both temperature and [CO_2], with elevated temperature having the major effect. Consequently, in addition to coarse control, both growth parameters appeared able to exert fine control over Rubisco activity, through activation of the enzyme.

Decreased total Rubisco activity in dark-sampled leaves indicates that rice and soybean belong to the group of plants that have enzyme activity regulated by CA1P. For several plant species, CA1P is synthesized relatively slowly over several hours at low irradiance or in the dark (Salvucci 1989; Seemann *et al.* 1990). Elevated growth [CO_2] and temperature appeared to have some influence on the metabolism and regulatory role of CA1P, and its effect might be species-specific. In rice, fine control of Rubisco activity, apparently mediated by CA1P, was affected by elevated [CO_2] but only marginally by high growth temperatures. In soybean, however, the reverse appeared to be the case in that elevated temperature rather than [CO_2] caused the greater response. This is similar to the greater effects of elevated temperature than [CO_2] on the protein, activation and apparent K_{cat} of Rubisco in soybean.

The data in this study demonstrate that, during growth, temperature and [CO_2] may have interactive effects on leaf photosynthesis. Elevated [CO_2] can compensate for adversely high growth temperatures in both rice and soybean in terms of net photosynthetic rates. However, at any given temperature the degree of leaf photosynthesis enhancement by a doubling of [CO_2] appears to be influenced by the temperature optimum for the species, the extent to which Rubisco is down-regulated, and by as yet unidentified species-specific differences. Both elevated temperature and [CO_2] exert coarse and fine control over Rubisco activity, with rice being more susceptible to down-regulation of this enzyme than soybean.

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