

Contrasting effects of carbon dioxide and irradiance on the acclimation of photosynthesis in developing soybean leaves

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Abstract

Leaves developed at high irradiance (I) often have higher photosynthetic capacity than those developed at low I , while leaves developed at elevated CO_2 concentration $[\text{CO}_2]$ often have reduced photosynthetic capacity compared with leaves developed at lower $[\text{CO}_2]$. Because both high I and elevated $[\text{CO}_2]$ stimulate photosynthesis of developing leaves, their contrasting effects on photosynthetic capacity at maturity suggest that the extra photosynthate may be utilized differently depending on whether I or $[\text{CO}_2]$ stimulates photosynthesis. These experiments were designed to test whether relationships between photosynthetic income and the net accumulation of soluble protein in developing leaves, or relationships between soluble protein and photosynthetic capacity at full expansion differed depending on whether I or $[\text{CO}_2]$ was varied during leaf development. Soybean plants were grown initially with a photosynthetic photon flux density (PPFD) of $950 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $350 \mu\text{mol}[\text{CO}_2] \text{mol}^{-1}$, then exposed to $[\text{CO}_2]$ ranging from 135 to $1400 \mu\text{mol mol}^{-1}$ for the last 3 d of expansion of third trifoliolate leaves. These results were compared with experiments in which I was varied at a constant $[\text{CO}_2]$ of $350 \mu\text{mol mol}^{-1}$ over the same developmental period. Increases in area and dry mass over the 3 d were determined along with daily photosynthesis and respiration. Photosynthetic CO_2 exchange characteristics and soluble protein content of leaves were determined at the end of the treatment periods. The increase in leaflet mass was about 28 % of the dry mass income from photosynthesis *minus* respiration, regardless of whether $[\text{CO}_2]$ or I was varied, except that very low I or $[\text{CO}_2]$ increased this percentage. Leaflet soluble protein per unit of area at full expansion had the same positive linear relationship to photosynthetic income whether $[\text{CO}_2]$ or I was varied. For variation in I , photosynthetic capacity varied directly with soluble protein per unit area. This was not the case for variation in $[\text{CO}_2]$. Increasing $[\text{CO}_2]$ reduced photosynthetic capacity per unit of soluble protein by up to a factor of 2.5, and photosynthetic capacity exhibited an optimum with respect to growth $[\text{CO}_2]$. Thus CO_2 did not alter the relationship between photosynthetic income and the utilization of photosynthate in the net accumulation of soluble protein, but did alter the relationship between soluble protein content and photosynthetic characteristics in this species.

Additional key words: dry matter accumulation; feedback inhibition; *Glycine max*; protein; photosynthetic CO_2 and O_2 exchange; ribulose-1,5-bisphosphate carboxylase/oxygenase; source-sink balance.

Introduction

One of the major uncertainties in predicting responses of plants to the increasing concentration of carbon dioxide in the atmosphere is the current inability to predict the extent of acclimation of photosynthesis to elevated $[\text{CO}_2]$. Acclimation of photosynthesis to growth at elevated $[\text{CO}_2]$ such that net photosynthetic rates, P_N , measured at a standard $[\text{CO}_2]$ are reduced in comparison with plants grown at lower $[\text{CO}_2]$, is a common but not

universal response in C_3 plants. Acclimation to elevated $[\text{CO}_2]$ can be sufficient to eliminate increases in P_N at the growth $[\text{CO}_2]$. Reasons for variation in the occurrence of acclimation are not known, but acclimation to elevated $[\text{CO}_2]$ may occur when plants are unable to fully utilize the additional photosynthate produced. An attractive possible explanation is that surplus photosynthate suppresses the expression of genes encoding some

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components of the photosynthetic apparatus (Sheen 1994), and results in reductions in the mRNA concentrations and enzyme activities of several photosynthetic components (e.g., ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBPCO; e.g., Krapp *et al.* 1993, Jang and Sheen 1994, Van Oosten *et al.* 1994, Van Oosten and Besford 1995). However, this raises the question as to why high I and cool temperatures, that also increase the production of photosynthate relative to the capacity of the plant to utilize it, generally increase the amounts of photosynthetic components per unit of leaf area and photosynthetic capacity (Berry and Björkman 1980, Bunce 1983), while elevated $[\text{CO}_2]$ decreases them. We hypothesized that increased I and $[\text{CO}_2]$ may have different effects on the utilization of the extra photosynthate in the development of the photosynthetic system. These experiments were designed to test whether $[\text{CO}_2]$ differs from I in its effect on the relationship between photosynthate supply and the net accumulation of soluble protein or on the relationship between soluble protein and photosynthetic characteristics. Experiments by Morin *et al.* (1992) on clover indicated that increased I and $[\text{CO}_2]$ had different effects on carbon partitioning

into saccharide fractions, but did not measure protein content, and measured P_N only on a whole plant basis. We are unaware of any direct comparisons of the effects of increased I and $[\text{CO}_2]$ on leaf soluble protein content or on photosynthetic characteristics.

The experiments were carried out using a previously developed soybean experimental system (Bunce 1991), in which half-expanded third trifoliolate leaves are exposed to various environmental conditions for the last 3 d of expansion, and photosynthetic characteristics assayed immediately after, at full expansion. Advantages of utilizing this system are that leaves at the half-expanded stage no longer import saccharides, so that the availability of photosynthate can be measured by CO_2 exchange and mass balance, and yet their photosynthetic capacity at full expansion is responsive to the environmental conditions during the last 3 d of expansion (Bunce 1991). Responses of P_N to variation in photosynthate supply as controlled by I at constant $[\text{CO}_2]$ were compared with responses where photosynthate supply was controlled by $[\text{CO}_2]$ at high I . Additionally, it was investigated whether the occurrence of acclimation to elevated $[\text{CO}_2]$ could be eliminated by reducing photosynthate supply by decreasing I .

Materials and methods

Soybean (*Glycine max* [L.] Merr. cv. Kent) was grown from seed in a controlled environment chamber. Chamber $[\text{CO}_2]$ was measured continuously with an infrared analyzer that controlled the injection of CO_2 or CO_2 -free air. Daytime $[\text{CO}_2]$ was 345 to 355 $\mu\text{mol mol}^{-1}$, and nighttime $[\text{CO}_2]$ was 350 to 380 $\mu\text{mol mol}^{-1}$. Chamber air temperature was 25 ± 0.2 °C, and the dew point temperature was 18 ± 1 °C. Plants were grown in plastic pots containing 1 800 cm^3 of vermiculite and were flushed daily with 500 cm^3 of a complete nutrient solution containing 13.5 mM nitrogen (Robinson 1984). Irradiance from high pressure sodium and metal halide lamps provided 950 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD) for 12 h per day.

On the morning of the third day after third mainstem trifoliolate leaves had unfolded (day 20 from planting), the plants were moved into a similar controlled environment chamber where various PPFD and $[\text{CO}_2]$ treatments were applied for 3 d. The chamber volume was about 1 m^3 , and the chamber was flushed continually with CO_2 -free air at a flow rate of 833 $\text{cm}^3 \text{s}^{-1}$ for all treatments, to allow $[\text{CO}_2]$ to be controlled at concentrations below that of room air. Chamber $[\text{CO}_2]$ was measured continuously with an infrared analyzer that controlled the injection of CO_2 to maintain the desired concentration. Lengths and widths of the

terminal leaflets of third trifoliolates were measured daily at lights on and at lights off. A regression between leaflet area and length times width ($r^2 = 0.998$) was used to estimate leaflet area at these time points. On the morning of the fourth day, the plants were returned to the initial chamber, and photosynthetic characteristics (see later) were measured after a few hours of irradiation. Terminal leaflets of third leaves of extra plants were harvested at the time plants were moved into the treatment chamber for determination of area and dry mass. Discs were removed for determination of soluble protein content, frozen in liquid nitrogen, and stored at -80 °C until analyzed. A similar harvest was made on leaves of treated plants immediately after photosynthetic characteristics were measured. Measurements of P_N at the growth conditions were made at 1, 6, and 11 h after lights on during each day, and 1 h after the beginning and 1 h before the end of each night during the treatment periods. These measurements were made on terminal leaflets of the third trifoliolate leaves of each plant, using a CIRAS-1 portable photosynthesis system (PP Systems, Haverhill, MA, USA). The daily sum of photosynthesis minus night respiration, multiplied by the leaflet area on each day was used to indicate the potential increase in dry mass, assuming a carbon content of 40 %. Four to six plants were given experimental treatments at one time.

and for each batch of treated plants, control plants were left in the original growth conditions. Each experimental growth condition was repeated at least twice.

P_N was measured at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, at substomatal CO₂ concentrations (C_i) of 220 ± 5 or 440 ± 10 $\mu\text{mol mol}^{-1}$, and the apparent quantum yield of photosynthesis at an external [CO₂] of 350 $\mu\text{mol mol}^{-1}$ based on the slope of the response of P_N to incident four PPFD between 80 and 180 $\mu\text{mol m}^{-2} \text{s}^{-1}$. All measurements were made with a CIRAS-1 system, at a leaf temperature of 25 °C, and a water vapor pressure deficit of less than 1.5 kPa. For a subset of the treatments (see Results), measurements of photosynthetic O₂ release at a [CO₂] of 20 mmol mol⁻¹ were made using a leaf disc oxygen electrode (Delieu and Walker 1981) to determine if the CO₂ exchange values were affected by patchy stomatal closure (Buckley *et al.* 1997). For another subset of the treatments, including those producing the widest range of P_N per unit of soluble protein, RuBPCO initial and fully activated activity and RuBPCO quantity were also determined, using the methods described in

Sicher and Bunce (1997).

The [CO₂] treatments applied were 1400, 700, 500, 350, 260, 200, and 135 $\mu\text{mol mol}^{-1}$ for 24 h per day. The PPFD for these [CO₂] treatments was 950 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 12 h per day. At a constant [CO₂] of 350 $\mu\text{mol mol}^{-1}$, plants were grown at constant PPFDs of 950, 730, 500, 370, and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and variable PPFDs of 370/730 and 150/950 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with the lower PPFD provided for the first and last 3 h of each day. Additionally, at a [CO₂] of 700 $\mu\text{mol mol}^{-1}$, plants were treated with constant PPFDs of 370 and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Similar values for the same I have been previously published (Bunce 1991), but the PPFD treatments were repeated for this study to allow more direct comparison with the [CO₂] treatments. The prior work indicated that growing plants initially at PPFDs of 550 and 950 $\mu\text{mol m}^{-2} \text{s}^{-1}$ produced similar results for the PPFD treatments, and also that the treatment PPFD of 950 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was saturating with respect to P_N and protein content (Bunce 1991).

Results

Neither the rate of increase in area nor the final areas were significantly affected by the PPFD or [CO₂] treatments. Leaflets from all treatments increased in area during the final day of treatment, but not during the final night. P_N measured under the growth conditions increased with growth [CO₂], despite evidence of a reduction in P_N (see later) when measured under

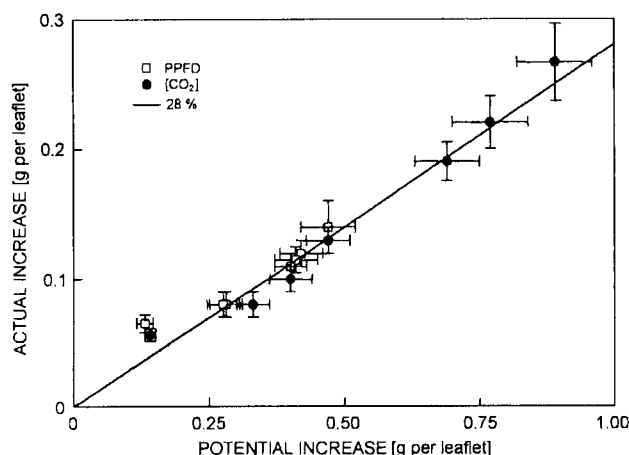


Fig. 1. The relationship between the potential increase in dry mass calculated from photosynthesis *minus* respiration, and the actual increase in mass of soybean leaflets exposed to a range of PPFD (*squares*) or [CO₂] (*circles*) treatments for three days. Each symbol represents the mean for 8 to 10 leaflets, with SE shown by bars. The line indicates an increase in mass equal to 28 % of the potential increase.

standardized conditions. Increasing PPFD and increasing [CO₂] both increased P_N *minus* night respiration per leaflet summed for the 3 d period, which represents the potential increase in mass. The actual increase in dry mass averaged 28 % (range of 25 to 31 % across treatments) of this potential value for all growth conditions except at the lowest values of potential income, where the percentage was 40 ± 5 % (SE) and 50 ± 6 % (SE) for low [CO₂] and low PPFD, respectively (Fig. 1). The PPFD and [CO₂] treatments produced the same relationship between potential and actual dry mass gain (Fig. 1), although the [CO₂] treatments extended to higher values of potential income. Analysis of covariance indicated that neither slope nor intercept of the linear regressions differed between PPFD and [CO₂] treatments at $p = 0.05$. There was also a positive linear relationship between the increase in dry mass over the 3-d treatment period and the mass of soluble protein per unit of area at full expansion (Fig. 2), and analysis of covariance indicated that the same relationship occurred for both the PPFD and the [CO₂] treatments. The mass of soluble protein per unit of area at full expansion increased with total dry mass per area and ranged from 8.7 to 11.4 % of the total dry mass across treatments, with a mean of 10.7 % (Fig. 2). The amount of soluble protein per leaflet at the end of the treatment period was 1.5 times that initially present, for the leaflets with the lowest protein content at the end of the treatment period.

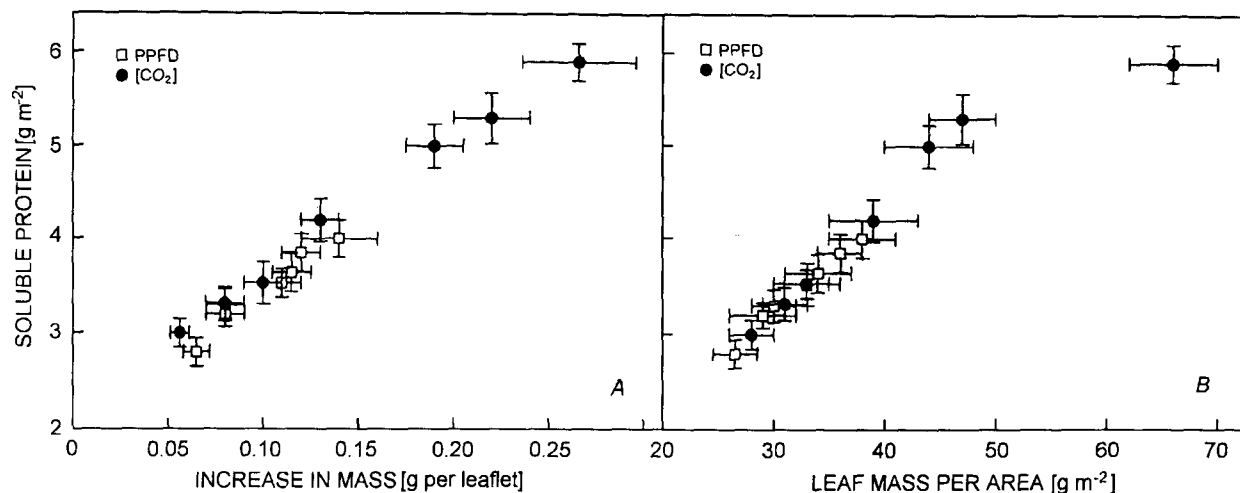


Fig. 2. Relationships between total soluble protein per unit of area at full expansion and (A) the actual increase in mass of leaflets or (B) dry mass per unit of area over three-day treatments of PPFD (squares) or $[\text{CO}_2]$ (circles). Each symbol represents the mean for 8 to 10 leaflets, with SE shown by bars.

For variation in PPFD, values of P_N per unit area measured at a C_i of $220 \mu\text{mol mol}^{-1}$ increased linearly with soluble protein per unit of area (Fig. 3A). However, for the $[\text{CO}_2]$ treatments, maximum P_N occurred at an intermediate value of soluble protein (Fig. 3A). Below this optimum value, low $[\text{CO}_2]$ treatments produced higher P_N than low PPFD treatments for a given mass of protein per unit area (Fig. 3A). Values of P_N at a C_i of $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ expressed per unit of soluble protein were nearly constant (*i.e.*, within 5 % and not significantly different by ANOVA) for variation in soluble protein per area caused by the PPFD treatments, but decreased significantly with increasing soluble protein for the $[\text{CO}_2]$ treatments (Fig. 3B). Analysis of covariance indicated that the PPFD and $[\text{CO}_2]$ treatments differed by a factor of 5 in the slope of the response of P_N per unit of soluble protein to soluble protein per unit of area, with the slopes significantly different at $p = 0.001$ (Fig. 3B). This comparison of slopes was not affected by inclusion of the values with protein contents of 4.5 g m^{-2} or more for the $[\text{CO}_2]$ treatments. P_N measured at a C_i of $440 \mu\text{mol mol}^{-1}$ also increased linearly with soluble protein per unit of area for the PPFD treatments, but exhibited an optimum at an intermediate value of soluble protein per area for the $[\text{CO}_2]$ treatments (Fig. 3C). No difference between the $[\text{CO}_2]$ and PPFD treatments was evident at low values of protein per area (Fig. 3C). P_N at $C_i = 440 \mu\text{mol mol}^{-1}$ expressed per unit of soluble protein were constant within 10 % (and not significantly different by ANOVA) for all soluble protein contents per area for the PPFD treatments, but decreased significantly with increasing soluble protein for the $[\text{CO}_2]$ treatments (Fig. 3D). Analysis of covariance indicated that the PPFD and $[\text{CO}_2]$ treatments differed by a factor of 2.9 in the slope of the response of P_N per unit of protein to

Table 1. Change in dry mass over the treatment period [g per leaflet], and dry mass [g m^{-2}], protein [g m^{-2}], and photosynthetic characteristics [$\mu\text{mol m}^{-2} \text{s}^{-1}$ or $\text{mmol kg}^{-1}(\text{protein}) \text{s}^{-1}$] at full expansion for third trifoliate leaves of soybean exposed for the last three days of expansion to either 350 or 700 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ at three different photosynthetic photon flux densities (PPFD) [$\mu\text{mol m}^{-2} \text{s}^{-1}$]. Values of means within a column and $[\text{CO}_2]$ treatment [$\mu\text{mol mol}^{-1}$] followed by different letters were significantly different at $p = 0.05$, based on $n = 8$ to 10.

PPFD	$[\text{CO}_2]$	Change in mass	Mass per area	Protein per area	P_N per area	P_N per protein
950	700	0.77 a	47 a	5.3 a	24.0 b	4.5 b
	350	0.47 b	38 b	4.0 b	29.7 a	7.4 a
370	700	0.42 a	37 a	4.1 a	24.2 a	5.9 a
	350	0.28 b	29 b	3.2 b	25.3 a	7.9 a
150	700	0.18 a	32 a	3.5 a	21.6 a	6.2 b
	350	0.13 b	26 b	2.8 b	22.1 a	7.9 a

protein per unit of area, with the slopes significantly different at $p = 0.002$ (Fig. 3D). Exclusion of the values with protein contents of 4.5 g m^{-2} or more for the $[\text{CO}_2]$ treatments increased the difference in slope between the PPFD and $[\text{CO}_2]$ treatments. For P_N measured at either $C_i = 220$ or $440 \mu\text{mol mol}^{-1}$, highest rates occurred at an intermediate value of growth $[\text{CO}_2]$ (Fig. 4). Apparent quantum yields of photosynthesis were not significantly affected by either the PPFD or $[\text{CO}_2]$ treatments, and averaged $0.042 \text{ mol mol}^{-1}$ at an external $[\text{CO}_2]$ of $350 \mu\text{mol mol}^{-1}$ (not shown).

When the 700 and $350 \mu\text{mol mol}^{-1}$ $[\text{CO}_2]$ treatments were compared at three growth PPFDs, the reduction in

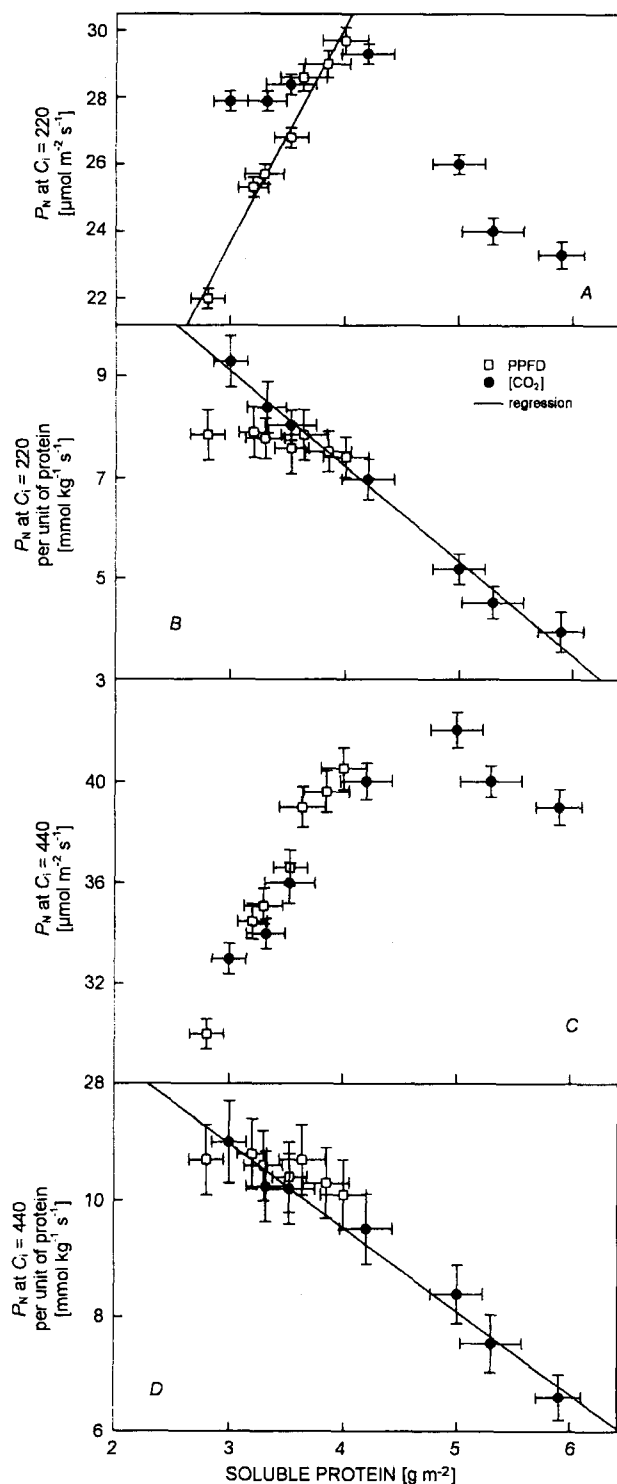


Fig. 3. Net photosynthetic rates (P_N) measured at $C_i = 220$ (A, B) or 440 (C, D) $\mu\text{mol mol}^{-1}$ as a function of soluble protein per unit of leaf area for soybeans leaflets exposed to various PPFD (squares) or [CO₂] (circles) treatments. Each symbol represents the mean for 8 to 10 leaflets, with SE shown by bars. The line represents the linear regression for the PPFD (A) or [CO₂] (B, D) treatments. In B, the equation of this line for the [CO₂] values is $P_N = 14.5 \pm 1.3 - (1.82 \pm 0.25 \text{ protein})$, with

$r^2 = 0.964$; for the PPFD treatment, the regression is $P_N = 9.0 \pm 0.5 - (0.37 \pm 0.20 \times \text{protein})$, with $r^2 = 0.482$, which is not significant at $p = 0.05$. In D, the equation of this line for the [CO₂] values is $P_N = 15.3 \pm 0.4 - (1.44 \pm 0.09 \text{ protein})$, with $r^2 = 0.981$; for the PPFD treatment, the regression is $P_N = 12.2 \pm 0.6 - (0.49 \pm 0.25 \times \text{protein})$, with $r^2 = 0.49$, which is not significant at $p = 0.05$.

P_N at $C_i = 220 \mu\text{mol mol}^{-1}$ caused by the higher [CO₂] was significant at the highest PPFD, but was not significant at the two lower PPFDs (Table 1). However, at each growth PPFD, the higher [CO₂] treatment increased photosynthetic income, total mass per area, and soluble protein at full expansion (Table 1). P_N values expressed per unit of soluble protein were lower at elevated [CO₂] at all growth PPFDs (Table 1).

The activity and quantity of RuBPCO per unit of area was determined for leaves of plants exposed to the 135, 350, 700, and 1400 $\mu\text{mol}[\text{CO}_2] \text{mol}^{-1}$ treatments at a PPFD of 950 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which includes the most extreme [CO₂] treatments. Enzyme activity per unit of area did not differ between these treatments, while both total soluble protein and RuBPCO protein per unit of area increased with increasing [CO₂] (Table 2). RuBPCO activity per unit of RuBPCO protein was highest for the lowest [CO₂] treatment (Table 2). RuBPCO protein decreased from 48 to 40 % of the soluble protein with increasing [CO₂].

Table 2. Soluble protein content and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) content and fully activated RuBPCO activity at full expansion for third trifoliolate leaves of soybean exposed to a range of [CO₂] [$\mu\text{mol mol}^{-1}$] for the last three days of expansion. Initial RuBPCO activity was 75 ± 2 % of the fully activated values, for all treatments. Values within a column followed by different letters were significantly different at $p = 0.05$, for $n = 8$.

[CO ₂]	Soluble protein	RuBPCO activity	Protein	spec. act.
135	3.3 d	46.6 a	1.60 c	29.1 a
350	4.2 c	43.5 a	1.83 bc	23.8 b
700	5.3 b	42.2 a	2.10 ab	20.1 b
1400	5.9 a	48.1 a	2.34 a	20.6 b

Photosynthetic O₂ exchange rates at saturating [CO₂] were determined for leaves treated at 350 and 700 $\mu\text{mol mol}^{-1}$ [CO₂] at 950 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. The rates did not differ significantly between [CO₂] treatments, with means of 47 and 44 $\mu\text{mol}(\text{O}_2) \text{m}^{-2} \text{s}^{-1}$ for the 350 and 700 $\mu\text{mol mol}^{-1}$ treatments, respectively. The similar rates occurred despite higher soluble protein in the leaves treated at the higher [CO₂] (Table 1). Rates of CO₂ assimilation measured at $C_i = 440 \mu\text{mol mol}^{-1}$ in the same leaves were 38 and 37 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ for the 350 and 700 $\mu\text{mol mol}^{-1}$ treatments, respectively.

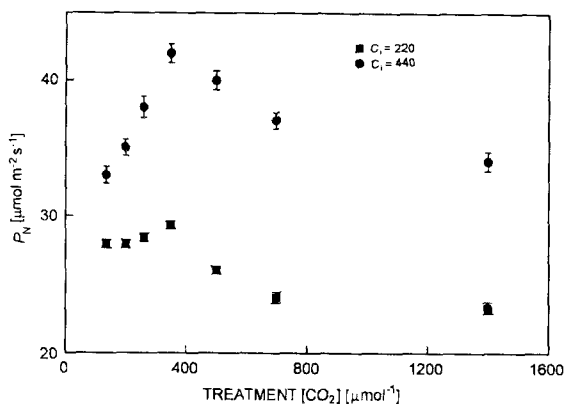


Fig. 4. Net photosynthetic rates (P_N) at full expansion measured at $C_i = 220$ (squares) or 440 (circles) $\mu\text{mol mol}^{-1}$ as a function of the $[\text{CO}_2]$ during the three day treatment period just prior to full expansion. Each symbol represents the mean for 8 to 10 leaflets, with SE shown by bars.

Discussion

The results indicated that the higher the photosynthetic income over the last three days of leaf expansion, the higher was the soluble protein content per unit of area at full expansion for both the PPFD and $[\text{CO}_2]$ treatments. There was no indication that PPFD and $[\text{CO}_2]$ had different effects on the net accumulation of soluble protein. High availability of photosynthate caused either by high PPFD or high $[\text{CO}_2]$ increased the accumulation of total soluble protein, as well as RuBPCO protein. This is contrary to the response expected for saccharide repression of gene expression. However, saccharides were not measured here, because other workers have so far failed to identify any specific saccharide pool consistently related to the repression of genes of photosynthetic enzymes (e.g., Moore *et al.* 1997, 1998). Our results support the idea of a positive feedback between photosynthate availability and photosynthetic capacity (Chabot *et al.* 1979, Bunce 1983, 1991), whereby environmental conditions which increase either P_N or reduce sink activity (e.g., low temperature) result in plants developing leaves with increased amounts of photosynthetic machinery per unit of leaf area.

Similar to the positive feedback between availability of photosynthate and photosynthetic capacity reported here for soybean, growth of barley at low temperatures increased both hexose concentrations and photosynthetic capacity (Holaday *et al.* 1992). A positive feedback between saccharide supply and photosynthetic capacity has also been observed in experiments in which sugars have been supplied to plants developing in tissue culture (Kovtun and Daie 1995, Furbank *et al.* 1997). These workers suggested that failure to find evidence of saccharide repression of photosynthetic gene expression in their experiments could be due to opposite responses depending upon developmental stage (Kovtun and Daie 1995), or to failure to reach a threshold saccharide concentration necessary for repression. However, repression of photosynthetic genes occurred in tomato leaves even early in development (Van Oosten and

Besford 1995), and in our study the supply of photosynthate was increased above the content available to plants grown at high PPFD at ambient $[\text{CO}_2]$, with no evidence of repression of RuBPCO content. These results apply only to this model system and do not contradict other cases of saccharide repression of gene expression. Nevertheless, they do not support our hypothesis that high I and $[\text{CO}_2]$ have different effects on the utilization of extra photosynthate in the net accumulation of soluble protein in developing soybean leaves.

While increased availability of photosynthate increased total soluble protein and RuBPCO protein per unit of area for both I and $[\text{CO}_2]$ treatments, the relationship of these to photosynthetic characteristics differed between the I and $[\text{CO}_2]$ treatments. For variation in I , P_N at both $C_i = 220$ and $440 \mu\text{mol mol}^{-1}$ increased linearly with increasing soluble protein per unit of area, and P_N expressed per unit of soluble protein were essentially constant across the range of soluble protein contents. This is the response expected if soluble protein (particularly RuBPCO) limits P_N . In contrast, for the $[\text{CO}_2]$ treatments, P_N expressed per unit of soluble protein decreased with increasing soluble protein per unit of area even at the rate limiting C_i of $220 \mu\text{mol mol}^{-1}$ and even at low soluble protein contents. For the $[\text{CO}_2]$ treatments, maximum P_N occurred at intermediate values of soluble protein. While it can not be ruled out that a similar pattern might occur at ambient $[\text{CO}_2]$ for treatments producing higher soluble protein contents than occurred here, the I -treatments used here were saturating for soluble protein content. In these experiments, photosynthetic acclimation to $[\text{CO}_2]$ involves regulation of RuBPCO activity rather than content. The nature of this regulation is not known, but could involve the activation of the enzyme, the binding of inhibitors, or allocation of some RuBPCO to the role of a storage protein. RuBPCO protein was a smaller fraction of the soluble protein for the highest $[\text{CO}_2]$ treatments, suggesting some reallocation of soluble

protein away from RuBPCO may have occurred. The values obtained with the oxygen electrode indicate that an increase in the degree of patchy stomatal closure in plants grown at elevated [CO₂] is unlikely to have caused their lower P_N .

The occurrence of photosynthetic acclimation to elevated [CO₂] in dim *I* was only evident with concurrent measurements of soluble protein. However, these values indicate that greatly restricting photosynthate supply relative to demand with dim *I* did not eliminate photosynthetic acclimation to elevated [CO₂]. This agrees with the finding that acclimation to elevated [CO₂] may occur even in the deep shade of a forest understory (Osborne *et al.* 1997). It is very difficult to envision how source-sink balance could explain

acclimation to EC in circumstances such as these, when plants remain source-limited at EC.

In conclusion, changes in both PPFD and [CO₂] during soybean leaf development had the same effect on the amount of soluble and RuBPCO protein per unit of area at full expansion. P_N expressed per unit of soluble protein were constant for the PPFD treatments, but decreased with increasing growth [CO₂], even for a similar range of protein contents. This decrease with increasing growth [CO₂] occurred even at low growth PPFD, indicating that surplus photosynthate was not the signal causing photosynthetic acclimation to [CO₂] in this case. The biochemical cause of reduced P_N in plants grown at EC was reduced *in vivo* RuBPCO activity but not content.

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