

# Up-regulation of photosynthesis and sucrose metabolism enzymes in young expanding leaves of sugarcane under elevated growth CO<sub>2</sub>

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Received 2 March 2006; accepted 3 March 2006

Available online 31 March 2006

## Abstract

Midday leaf CO<sub>2</sub> exchange rate (CER), concentration of chlorophyll (Chl) and soluble protein and activity of the primary enzymes involved in leaf photosynthesis and sucrose metabolism were determined during leaf ontogeny for sugarcane plants grown at ambient (360 μmol mol<sup>-1</sup>) and double-ambient (elevated, 720 μmol mol<sup>-1</sup>) CO<sub>2</sub>. Although leaf CER of both CO<sub>2</sub> treatments increased and was highest at 14 days after leaf emergence (DALE), leaf CER of the elevated-CO<sub>2</sub> plants, however, was 20, 7 and 10% greater than that of the ambient-CO<sub>2</sub> plants at 7, 14 and 32 DALE, respectively. Elevated-CO<sub>2</sub> plants also had up to 51% lower stomatal conductance and 39% less transpiration, which resulted in 26–52% greater water-use efficiency (WUE) than ambient-CO<sub>2</sub> plants, during leaf growth and development. Concentrations of total Chl and soluble protein and activities of RuBP carboxylase-oxygenase (Rubisco), PEP carboxylase (PEPC), NADP-malate dehydrogenase (NADP-MDH), pyruvate P<sub>i</sub> dikinase (PPDK) and sucrose-P synthase (SPS), expressed on a leaf area basis, generally followed leaf CER patterns during leaf ontogeny. For the elevated-CO<sub>2</sub> plants, total Chl and soluble protein were 31 and 15% greater, and Rubisco, PPDK and NADP-MDH were up-regulated by 21, 117 and 174%, respectively, at 14 DALE, whereas PEPC and NADP-malic enzyme tended to be lower than or similar to the ambient-CO<sub>2</sub> plants throughout leaf development. In addition, leaf SPS activity was increased by 13 and 37% and leaf sucrose concentration was 31 and 19% higher at 7 and 14 DALE, respectively, under elevated growth CO<sub>2</sub>. At final harvest, elevated growth [CO<sub>2</sub>] enhanced leaf area by 31%, leaf fresh weight by 13.5%, stem fresh weight by 55.5%, total above-ground plant fresh weight by 44%, and stem juice volume by 83%. The up-regulation of the key photosynthesis and sucrose metabolism enzymes at early stages of leaf development would indicate an acclimation to elevated growth [CO<sub>2</sub>] for the C<sub>4</sub> sugarcane plant. An up-regulation of the enzymes, together with a reduction in leaf stomatal conductance and transpiration and an improvement in leaf WUE and plant water status, could lead to an enhancement in leaf area, plant biomass accumulation and sucrose production for the CO<sub>2</sub>-enriched sugarcane plants.

Published by Elsevier Ireland Ltd.

**Keywords:** Rising atmospheric CO<sub>2</sub>; C<sub>4</sub> photosynthetic enzymes; Sucrose metabolism

## 1. Introduction

As the present atmospheric carbon dioxide level ([CO<sub>2</sub>]), currently at about 375 μmol mol<sup>-1</sup>, limits photosynthesis and

growth of many plants, rising atmospheric [CO<sub>2</sub>] could potentially benefit agricultural crops [1,2]. In C<sub>3</sub> plants, the binding of atmospheric CO<sub>2</sub> to its primary acceptor, ribulose biphosphate (RuBP), is catalyzed in the mesophyll cells by Rubisco, and the product of this carboxylation reaction, 3-phosphoglycerate (PGA), is converted to other carbohydrates, including sucrose and starch. In addition, Rubisco is also capable of catalyzing an oxygenase reaction in which O<sub>2</sub> reacts with RuBP to form PGA and phosphoglycolate. The metabolism of phosphoglycolate and subsequent release of CO<sub>2</sub>, widely known as photorespiration, have an adverse effect on the photosynthetic efficiency of C<sub>3</sub> plants [3]. As the balance between carboxylation and oxygenation of RuBP depends on the relative concentration

*Abbreviations:* CER, CO<sub>2</sub> exchange rates; Chl, chlorophyll; DALE, days after leaf emergence; NADP-MDH, NADP-malate dehydrogenase; NADP-ME, NADP-malic enzyme; PEPC, phosphoenolpyruvate carboxylase; Rubisco, ribulose biphosphate carboxylase-oxygenase; SPS, sucrose phosphate synthase; WUE, water-use efficiency

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of CO<sub>2</sub> and O<sub>2</sub> at the Rubisco site, a higher atmospheric [CO<sub>2</sub>] reduces photorespiration and enhances photosynthetic CO<sub>2</sub> exchange rate (CER) and growth of C<sub>3</sub> plants [1].

C<sub>4</sub> plants have developed a CO<sub>2</sub>-concentrating mechanism to overcome the limitations of low atmospheric [CO<sub>2</sub>] and photorespiration. Leaves of C<sub>4</sub> plants feature a Kranz architecture, having both mesophyll cells where atmospheric CO<sub>2</sub> is fixed by phosphoenolpyruvate carboxylase (PEPC) into C<sub>4</sub> acids and bundle sheath cells in which Rubisco refixes the CO<sub>2</sub> released from the C<sub>4</sub> acids [4]. Thus, the reactions that are unique to C<sub>4</sub> photosynthesis can be considered as an additional step to the conventional C<sub>3</sub> pathway. They operate to transfer CO<sub>2</sub> from mesophyll cells to bundle sheath cells through the intermediary of dicarboxylic acids, and consequently increase the concentration of CO<sub>2</sub> in the bundle sheath cells specifically for refixation via Rubisco in the C<sub>3</sub> photosynthetic pathway. Through this additional step, C<sub>4</sub> plants are able to concentrate [CO<sub>2</sub>] at the Rubisco site to levels manifold higher than ambient [CO<sub>2</sub>] [5,6]. Photosynthesis by C<sub>4</sub> plants is therefore near saturation at current atmospheric [CO<sub>2</sub>], and a rise in atmospheric [CO<sub>2</sub>] presumably may have little impact on C<sub>4</sub> photosynthesis and growth.

As a result, research of rising atmospheric [CO<sub>2</sub>] on basic photosynthetic processes has focused mainly on C<sub>3</sub> species. Nevertheless, the literature does reveal a positive growth response of many C<sub>4</sub> plants to elevated [CO<sub>2</sub>], although to a smaller extent than C<sub>3</sub> plants [7–9]. Such increases in biomass are not easily explained, because these C<sub>4</sub> plants often show little or no enhancement in short-term CER measurements of fully expanded mature leaves at the elevated [CO<sub>2</sub>] used for growth, which is in contrast to the C<sub>3</sub> species [8–16].

Although C<sub>4</sub> plants represent a small proportion of the world's plant species, their ecological and economic significance is substantial. On a global basis, about one-fifth of gross primary productivity is provided by C<sub>4</sub> plants [17–19]. In many tropical regions, the food source is primarily based on C<sub>4</sub> species, which supply grains for human consumption and forage for livestock [20]. Maize, millet, sorghum and sugarcane are the most important C<sub>4</sub> world crops in terms of production [20]. As the photosynthetic mechanism of a plant species is the major determinant of how it will respond to changes in [CO<sub>2</sub>] [1], an understanding of the photosynthetic mechanisms as well as growth and development underlying the responses of economically important C<sub>4</sub> crops to rising atmospheric [CO<sub>2</sub>] remains a crucial area of interest, and is essential for improvement of crop plant growth and yield efficiency in a future climate-changed world.

In the present study, sugarcane was grown at ambient and double-ambient [CO<sub>2</sub>]. Leaf CER, concentration of chlorophyll and soluble protein and activity of the key enzymes involved in C<sub>4</sub> photosynthetic pathway and sucrose metabolism were determined at various periods during leaf ontogeny. Our objective was to characterize the leaf photosynthetic mechanism of sugarcane under double-ambient growth [CO<sub>2</sub>], and to test the hypothesis that leaf photosynthesis of this C<sub>4</sub> crop plant is responsive to elevated growth [CO<sub>2</sub>] at an early stage of leaf development.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

Sugarcane (*Saccharum officinarum* L. cv. CP73-1547) was grown in Gainesville, Florida (29°38'N and 82°22'W) under field-like conditions for sunlight in paired-companion, temperature-gradient greenhouses (TGGs). The structural characteristics, specific methods and quality of CO<sub>2</sub> and temperature controls in the TGGs were previously described in detail [21]. These TGGs, with semi-cylindrical plastic-covered arch structures 27.4-m long, 4.3-m wide and 2.2-m high at the ridgepole, provided technology to study a wide variety of agricultural plant species grown season-long under enriched [CO<sub>2</sub>] and a range of temperatures [21–23]. The transparent greenhouse polyethylene transmitted 90% of the solar photosynthetic photon flux density (PPFD), so that plants received direct, natural solar irradiance. Typically, for the day of 4 September 1998, a clear day, maximum PPFD measured at midday inside the greenhouse was 1805 μmol photons m<sup>-2</sup> s<sup>-1</sup> at plant height.

Sugarcane was first propagated vegetatively in February 1997 by placing 10-cm stalk cuttings (single-node stem cuttings containing a vegetative bud) in flats filled with a potting medium in a propagation greenhouse maintained at 30 °C. On 20 March 1997, homogeneous germinated plants were transplanted into galvanized metal containers, 1.5-m long × 0.6-m wide × 0.6-m deep, containing Arredondo fine sand, a local mineral topsoil similar to mineral soils that are used for part of Florida's sugarcane production [24]. Eight containers were arranged in each of the two TGGs. Daytime CO<sub>2</sub> concentration was maintained at 360 μmol mol<sup>-1</sup> (ambient) in one TGG, and 720 μmol mol<sup>-1</sup> (double-ambient, elevated) in the other. The CO<sub>2</sub> values of 360 and 720 μmol mol<sup>-1</sup> were set to allow comparing the current experiment to [CO<sub>2</sub>] treatments in experiments performed with other crop plants in the mid 1990s when the value of "ambient" [CO<sub>2</sub>] was closer to 360 μmol mol<sup>-1</sup>. Soil moisture was checked daily, and additional irrigation applied as needed to ensure adequate soil moisture for plant growth. Fertilizers containing major and microelements were applied to the soil at time of transplanting, and biweekly during the growth season, at doses recommended for commercial sugarcane production in Florida, to provide optimum nutrient supply for plant growth [24]. On 24 June 1997, main stem stalks were harvested for biomass evaluation while small young tiller stalks were let continue to grow. A second harvest of all above-ground plant material was performed in December 1997. Regrowth of new tillers began in early spring 1998, and large stem stalks were harvested on 22 June, while small young tiller stalks (<15 cm of height) were let continue to grow through the rest of the year (i.e., from July to December). On 1 September 1998, plants having main stem stalks of similar size and height were selected. The newly emerged uppermost leaves (leaf 7) of the selected plants were then tagged. Plants were about 70-day-old at this growth stage. During the months of November to April and May to October, minimum/maximum temperatures of the

zones inside the TGGs where sugarcane plants were grown averaged 11.5 °C/24.5 °C and 20.1 °C/32.8 °C for 1997, and 11.4 °C/25.1 °C and 22.6 °C/35.1 °C for 1998, respectively.

## 2.2. Gas exchange measurements

CER, conductance and transpiration were determined at midday (1400–1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD) on outermost sections (near leaf tips) of the attached leaf blades (leaf 7), using a LI 6200 Photosynthesis System and LI 6000-12 (1-dm<sup>3</sup> volume) cuvette (LI-COR, Lincoln, NE), as previously reported [25]. Measurements were made at 7, 14, 32 and 48 days after leaf emergence (DALE).

## 2.3. Leaf sampling for biochemical analyses

Following gas exchange measurements, the outermost sections (near leaf tips) of the leaves that had been used for the photosynthetic determinations were detached from the leaf blades and immediately immersed in liquid N<sub>2</sub>. Sampled leaves were pooled by treatment, ground to a fine powder in liquid N<sub>2</sub>, and stored in liquid N<sub>2</sub> until analysis. Leaf fresh weight and area were also determined for a subset of plants at the same time of leaf sampling for biochemical analyses.

## 2.4. Assay of the enzymes and determination of the soluble sugars

From the liquid N<sub>2</sub> frozen leaf powder, activities of RuBP carboxylase-oxygenase (Rubisco), PEP carboxylase (PEPC), NADP-malate dehydrogenase (NADP-MDH), pyruvate P<sub>i</sub> dikinase (PPDK) and NADP-malic enzyme (NADP-ME) were assayed [26]. Sucrose phosphate synthase (SPS) and concentrations of sucrose and reducing sugars (glucose and fructose) were determined as previously reported [25]. Leaf chlorophyll (Chl) and soluble protein were extracted and concentrations were measured, as described [27,28].

## 3. Results

Midday CER and water-use efficiency (WUE), determined at 7, 14, 32 and 48 DALE on outermost sections (near leaf tips) of the leaf blades (leaf 7) of sugarcane grown at ambient (360  $\mu\text{mol mol}^{-1}$ ) and elevated (720  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub>, are shown in Fig. 1. Leaf CER, measured at the growth [CO<sub>2</sub>], was highest at 14 DALE for both ambient- and elevated-CO<sub>2</sub> plants (Fig. 1A). However, leaf CER of the elevated-CO<sub>2</sub> plants was 20% greater than that of the ambient-CO<sub>2</sub> plants at 7 DALE, and there was also a small stimulation of 7 and 10% at 14 and 32 DALE, respectively (Fig. 1A). During leaf development, elevated-CO<sub>2</sub> plants, when compared with ambient-CO<sub>2</sub> plants, had up to 51% lower stomatal conductance and 39% lower transpiration (data not shown), and 26–52% greater in WUE (Fig. 1B).

Concentrations of total Chl (Chl *a* + Chl *b*) and soluble protein and activities of the primary C<sub>4</sub> photosynthetic and sucrose metabolism enzymes, determined on outermost leaf

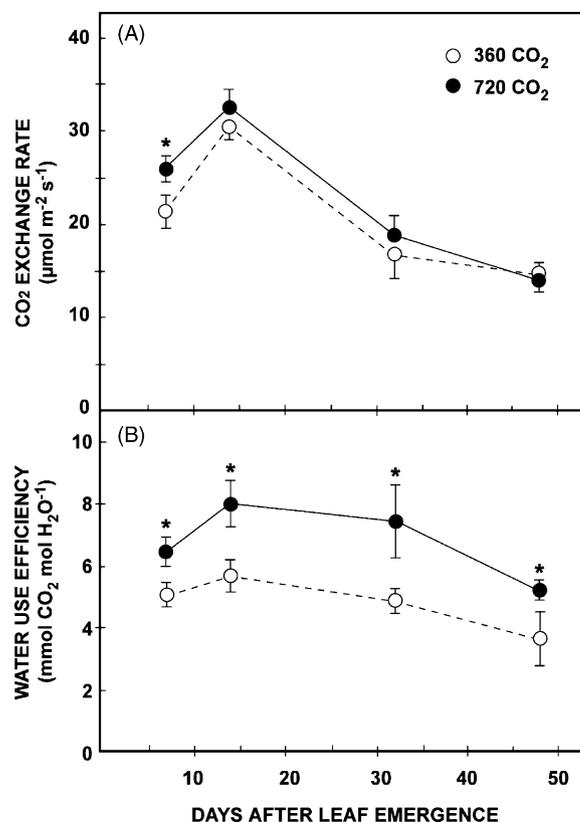


Fig. 1. Leaf CO<sub>2</sub> exchange rate (CER) (A) and water-use efficiency (WUE) (B) of sugarcane plants grown at ambient (○, 360  $\mu\text{mol mol}^{-1}$ ) and double-ambient (●, 720  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub>. Midday CER and WUE of outermost sections (near leaf tips) of leaf 7 were determined between 11:00 and 14:00 h (1400–1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD), at 7, 14, 32 and 48 days after leaf emergence. Values are the mean of 5–16 determinations. Vertical bars represent standard error of the mean. Asterisks indicate significant difference between the means at 5% level in a *t*-test.

sections (near leaf tips) of the leaf blades (leaf 7) at various DALE for the ambient- and elevated-CO<sub>2</sub> sugarcane plants, are shown in Figs. 2–5. Changes in concentrations of total Chl and protein and activities of the enzymes, expressed on a leaf area basis, generally correlated well with leaf CER during leaf ontogeny for both CO<sub>2</sub> treatments. At 14 DALE, concentrations of total Chl (Fig. 2A) and protein (Fig. 2B) of the CO<sub>2</sub>-enriched plants were 30 and 15% greater. Chlorophyll *a* made up 76–78% of total Chl concentration and the Chl *a*/Chl *b* ratio ranged from 3.2 to 3.6 for the various leaf growth stages for plants of both CO<sub>2</sub> treatments. In addition, patterns of changes in Chl *a* and Chl *b* concentrations during leaf growth and development (data not shown) were similar to those of total Chl for both CO<sub>2</sub> treatments. At 14 DALE, activities of Rubisco (Fig. 3A), PPDK (Fig. 4A) and NADP-MDH (Fig. 4B) were up-regulated by 21, 117 and 174%, respectively, for the elevated-CO<sub>2</sub> plants. In contrast, activity of PEPC (Fig. 3B) was 4–21% lower at all DALE, and that of NADP-ME (Fig. 5A) was 20–30% less at 7 and 14 DALE, respectively, under the CO<sub>2</sub>-enriched growth regime.

Leaf area, leaf fresh weight, stem fresh weight, total above-ground plant fresh weight and stem juice volume, determined at final harvest for the two CO<sub>2</sub> treatments, were all enhanced by

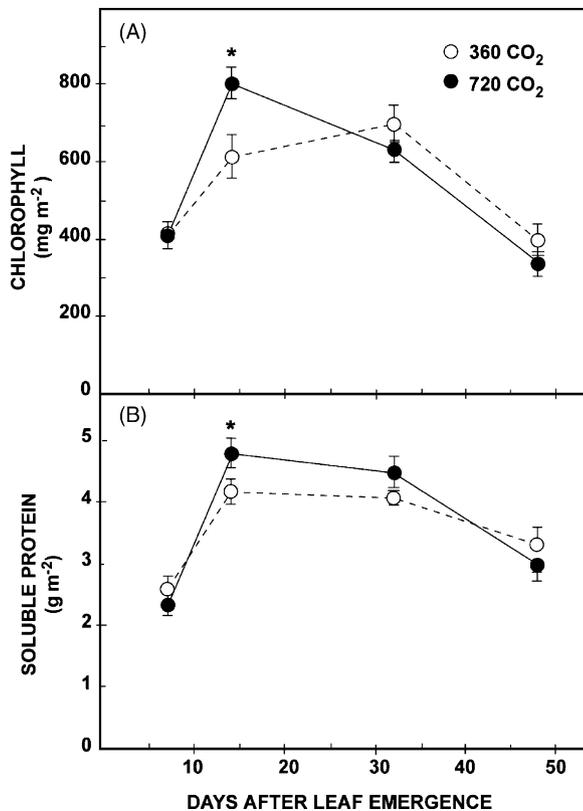


Fig. 2. Total leaf chlorophyll (A) and soluble protein (B) of sugarcane plants grown at ambient (○, 360  $\mu\text{mol mol}^{-1}$ ) and double-ambient (●, 720  $\mu\text{mol mol}^{-1}$ )  $\text{CO}_2$ . Outermost sections (near leaf tips) of leaf 7 were sampled at 7, 14, 32 and 48 days after leaf emergence, following midday CER measurements, and chlorophyll and soluble protein were extracted from the liquid  $\text{N}_2$  frozen leaf powder, as described in Section 2. Values are the mean of four determinations. Vertical bars represent standard error of the mean. Asterisks indicate significant difference between the means at 5% level in a *t*-test.

growth under elevated  $[\text{CO}_2]$  (Table 1). The enhancements for sugarcane grown under elevated  $[\text{CO}_2]$  were 31% in leaf area, 13.5% in leaf fresh weight, 55.5% in stem fresh weight, 44% in total above-ground plant fresh weight and 83% in stem juice volume, compared to companion plants grown at ambient  $[\text{CO}_2]$ .

Midday activity of SPS and concentration of soluble sugars for leaves of ambient and double-ambient  $\text{CO}_2$ -grown sugarcane plants are shown in Fig. 5B and Table 2, respectively.

Table 1

Leaf area, fresh weights of leaf, stem and total above-ground plant and stem juice volume of sugarcane plants grown at 360 and 720  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$

Growth parameters	$[\text{CO}_2]$	
	360 $\mu\text{mol mol}^{-1}$	720 $\mu\text{mol mol}^{-1}$
Leaf area ( $\text{dm}^2 \text{ plant}^{-1}$ )	34.1 $\pm$ 4.1 b	44.5 $\pm$ 2.1 a
Leaf fresh weight ( $\text{g plant}^{-1}$ )	165.0 $\pm$ 13.8 a	187.0 $\pm$ 6.4 a
Stem fresh weight ( $\text{g plant}^{-1}$ )	437.3 $\pm$ 48.0 b	680.3 $\pm$ 16.7 a
Above-ground ( $\text{g plant}^{-1}$ )	602.0 $\pm$ 60.0 b	867.0 $\pm$ 21.4 a
Juice ( $\text{cm}^3 \text{ main stem}^{-1}$ )	95.0 $\pm$ 18.4 b	174.3 $\pm$ 9.6 a

The growth parameters were determined at final harvest. Values are the mean  $\pm$  S.E. of four plants. Means within a row followed by different letters (a and b) are significantly different at 5% level in a *t*-test.

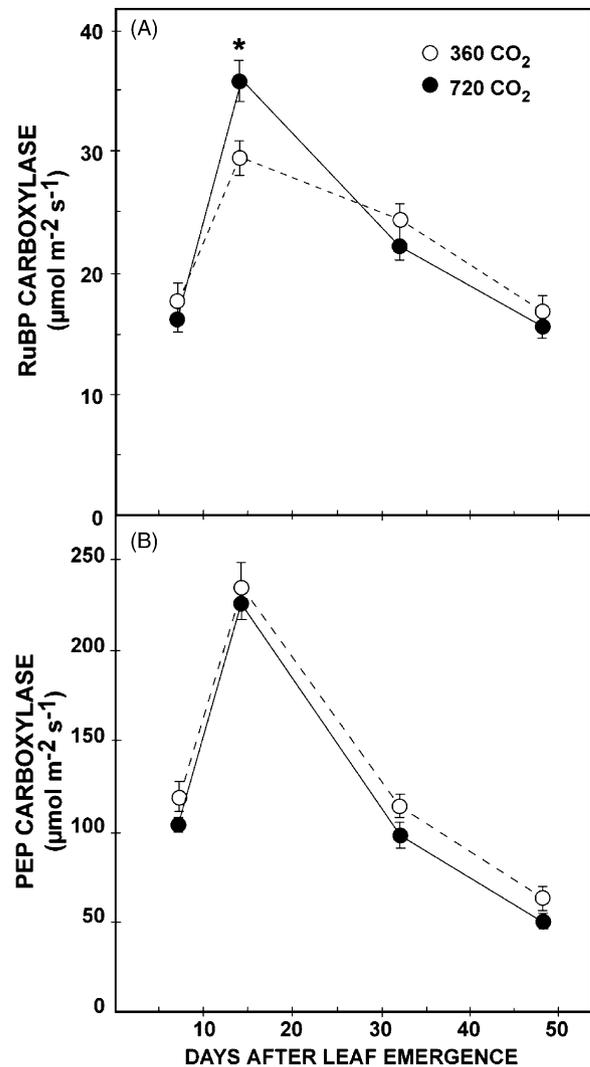


Fig. 3. Activities of RuBP carboxylase (A) and PEP carboxylase (B) of sugarcane plants grown at ambient (○, 360  $\mu\text{mol mol}^{-1}$ ) and double-ambient (●, 720  $\mu\text{mol mol}^{-1}$ )  $\text{CO}_2$ . Outermost sections (near leaf tips) of leaf 7 were sampled at 7, 14, 32 and 48 days after leaf emergence following midday leaf CER measurements. Activities of the enzymes were determined in extracts from the liquid  $\text{N}_2$  frozen leaf powder, as described in Section 2. Values are the mean of four determinations. Vertical bars represent standard error of the mean. Asterisks indicate significant difference between the means at 5% level in a *t*-test.

Activity of SPS in the elevated- $\text{CO}_2$  plants was increased by 13 and 37% at 7 and 14 DALE (Fig. 5B). Compared with the ambient- $\text{CO}_2$  plants, leaf sucrose concentration of the elevated- $\text{CO}_2$  plants was 31 and 19% greater, and concentration of total soluble sugars (sucrose + reducing sugars) was increased by 26 and 15% at 7 and 14 DALE, respectively (Table 2).

Figs. 6–9 show the data of leaf CER (Fig. 1) being expressed relative to the activity of each specific enzyme (Figs. 3–5) and to the concentration of total Chl and soluble protein (Fig. 2) for each  $[\text{CO}_2]$  treatment. There was relatively good correlation (*r*) of CER with activity of Rubisco (Fig. 6A), PEPC (Fig. 6B) and SPS (Fig. 8B) for both  $[\text{CO}_2]$  treatments. In contrast, the relationship was weak for PPDK (Fig. 7A), NADP-MDH (Fig. 7B), NADP-ME (Fig. 8A), and total Chl and soluble protein (Fig. 9).

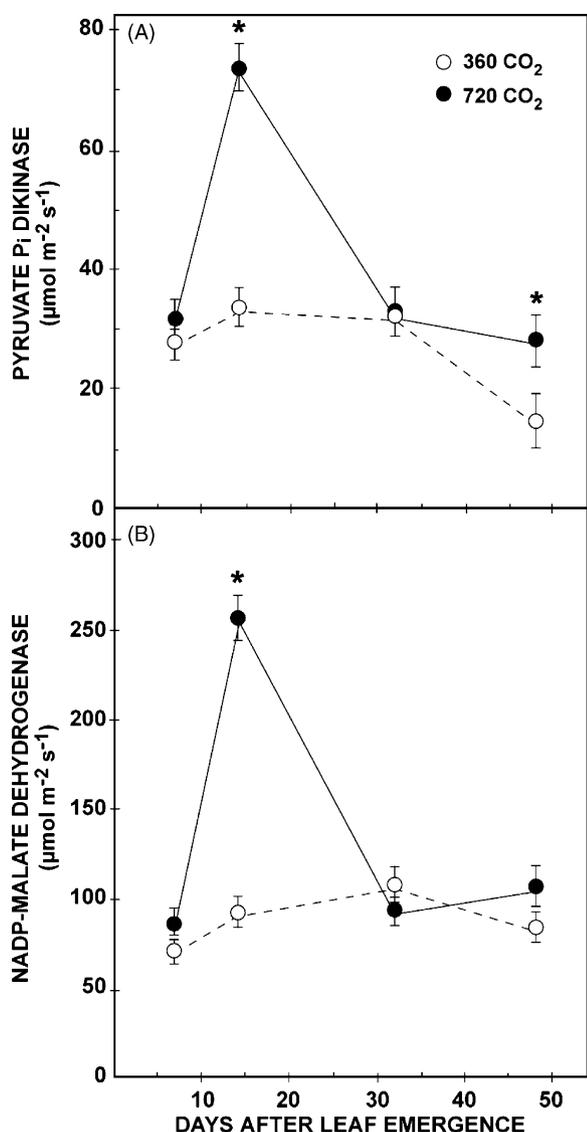


Fig. 4. Activities of pyruvate P<sub>i</sub> dikinase (A) and NADP-malate dehydrogenase (B) of sugarcane plants grown at ambient (○, 360 µmol mol<sup>-1</sup>) and double-ambient (●, 720 µmol mol<sup>-1</sup>) CO<sub>2</sub>. Outermost sections (near leaf tips) of leaf 7 were sampled at 7, 14, 32 and 48 days after leaf emergence following midday CER measurements, and enzyme activities were determined in extracts from the liquid N<sub>2</sub> frozen leaf powder as described in Section 2. Values are the mean of four determinations. Vertical bars represent standard error of the mean. Asterisks indicate significant difference between the means at 5% level in a *t*-test.

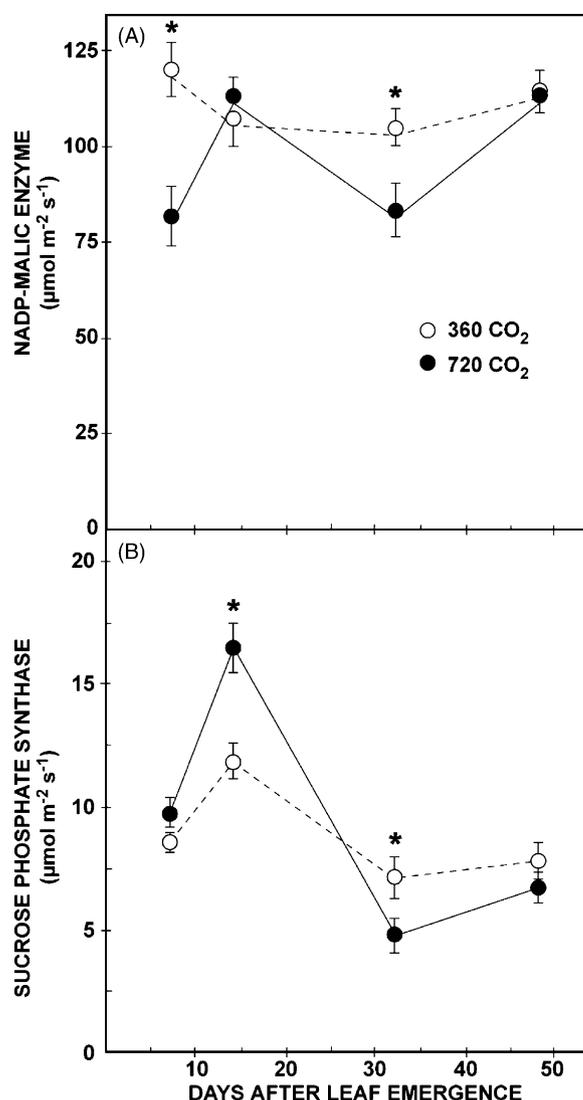


Fig. 5. Activities of NADP-malic enzyme (A) and sucrose phosphate synthase (B) of sugarcane plants grown at ambient (○, 360 µmol mol<sup>-1</sup>) and double-ambient (●, 720 µmol mol<sup>-1</sup>) CO<sub>2</sub>. Outermost sections (near leaf tips) of leaf 7 were sampled at 7, 14, 32 and 48 days after leaf emergence following midday CER measurements, and enzyme activities were determined in extracts from the liquid N<sub>2</sub> frozen leaf powder as described in Section 2. Values are the mean of four determinations. Vertical bars represent standard error of the mean. Asterisks indicate significant difference between the means at 5% level in a *t*-test.

Table 2

Concentrations of sucrose and soluble sugars in outermost sections of the leaf blades of sugarcane grown at 360 and 720 µmol mol<sup>-1</sup> CO<sub>2</sub>

[CO <sub>2</sub> ] (µmol mol <sup>-1</sup> )	DALE (days)	Sucrose (g m <sup>-2</sup> leaf area)	Total soluble sugars (g m <sup>-2</sup> leaf area)
360	7	1.83 ± 0.12 e	2.39 ± 0.18 e
	14	3.25 ± 0.15 b	3.83 ± 0.19 bc
	32	3.47 ± 0.20 ab	4.36 ± 0.28 ab
	48	2.73 ± 0.23 cd	3.33 ± 0.22 cd
720	7	2.39 ± 0.11 d	3.00 ± 0.18 d
	14	3.88 ± 0.26 a	4.42 ± 0.23 a
	32	3.33 ± 0.24 ab	4.22 ± 0.26 ab
	48	3.09 ± 0.12 bc	3.42 ± 0.25 cd

Leaves were sampled at midday, at 7, 14, 32 and 48 days after leaf emergence (DALE). Values are the mean ± S.E. of three determinations. Means with different letters (a–e) in the same column are significantly different at 5% level in a Duncan multiple range test.

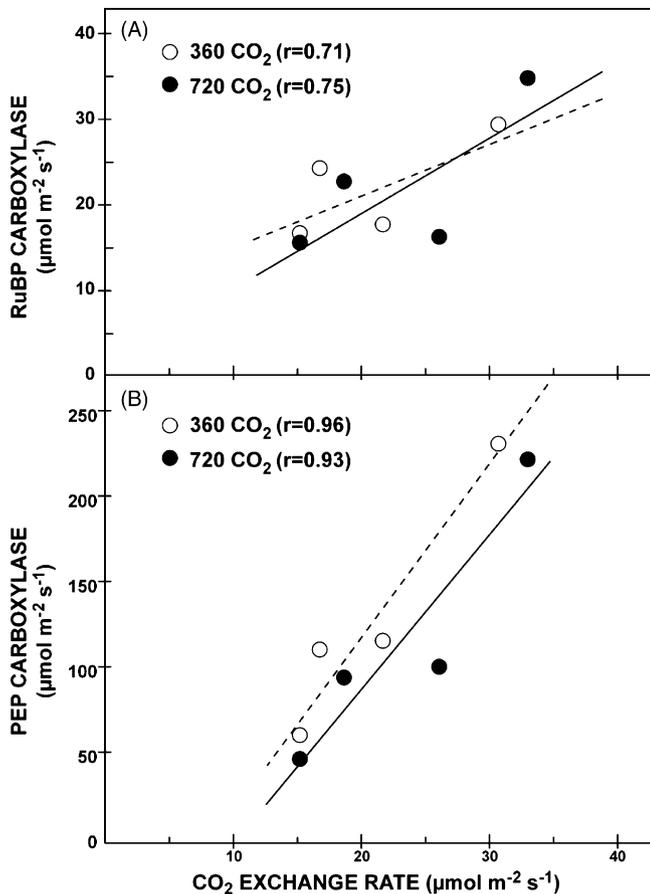


Fig. 6. CO<sub>2</sub> exchange rates (CER) vs. the activities of RuBP carboxylase (A) and PEP carboxylase (B) for outermost sections (near leaf tips) of leaf 7 of sugarcane plants grown at ambient (○, 360 μmol mol<sup>-1</sup>) and double-ambient (●, 720 μmol mol<sup>-1</sup>) CO<sub>2</sub>. Leaf CER and activities of the enzymes were determined at 7, 14, 32 and 48 days after leaf emergence. The solid line and broken line in each figure represent the best fitting lines for the double-ambient and ambient-CO<sub>2</sub> treatment, respectively, and the correlation coefficients (*r* values) are given in each figure.

#### 4. Discussion

Previous studies with sugarcane and some other C<sub>4</sub> monocots found a response in plant biomass to elevated growth [CO<sub>2</sub>] without the concomitant enhancement in leaf CER [11,13,29]. These short-term photosynthetic measurements, however, were carried out on fully expanded leaves. Recent studies show that leaf CER of these C<sub>4</sub> monocots, when measured at an early growth stage of the leaf or plant, is responsive to elevated [CO<sub>2</sub>]. In sorghum, elevated [CO<sub>2</sub>] enhances CER of young expanding leaves, but not mature leaves [30]. In maize, growth at elevated [CO<sub>2</sub>] also results in greater CER early in the growth season [31]. For sugarcane in the present study, the enhancement in CER at elevated-CO<sub>2</sub> was more apparent during early leaf development (Fig. 1). Furthermore, there has been indication that young developing leaf tissues of some C<sub>4</sub> crop plants may utilize a C<sub>3</sub>-like pathway for carbon fixation. In field-grown sorghum, Rubisco accumulates before PEPC is detected in the youngest tissues at the base of the leaf [32]. Similarly, there may be some direct entry of CO<sub>2</sub> into the C<sub>3</sub> pathway in the young leaf tissues

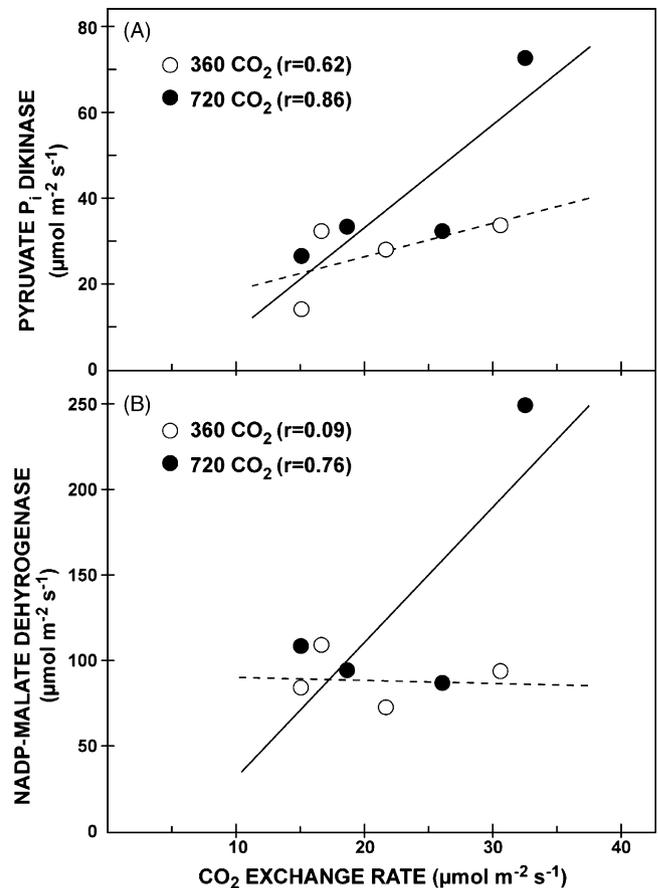


Fig. 7. CO<sub>2</sub> exchange rates (CER) vs. the activities of pyruvate P<sub>1</sub> dikinase (A) and NADP-malate dehydrogenase (B) for outermost sections (near leaf tips) of leaf 7 of sugarcane plants grown at ambient (○, 360 μmol mol<sup>-1</sup>) and double-ambient (●, 720 μmol mol<sup>-1</sup>) CO<sub>2</sub>. Leaf CER and activities of the enzymes were determined at 7, 14, 32 and 48 days after leaf emergence. The solid line and broken line in each figure represent the best fitting lines for the double-ambient and ambient-CO<sub>2</sub> treatment, respectively, and the correlation coefficients (*r* values) are given in each figure.

of maize [33]. As a consequence, young expanding leaves of sorghum and maize are likely more responsive to elevated growth [CO<sub>2</sub>] than mature leaves. In sugarcane, activities of a number of photosynthetic enzymes were up-regulated at an early growth stage of the leaf under double-ambient growth [CO<sub>2</sub>] (Figs. 3 and 4).

A positive growth response to elevated [CO<sub>2</sub>] has been reported for a number of C<sub>4</sub> plants [7–9,11,13,15,34,35]. The enhancement of C<sub>4</sub> biomass under elevated growth [CO<sub>2</sub>] has been typically attributed to a reduction in stomatal aperture and conductance and a subsequent improvement in leaf water status [1,2,15,34–38]. The reduction in stomatal conductance is a common response of plants to elevated growth [CO<sub>2</sub>] and occurs across a wide variety of both C<sub>3</sub> and C<sub>4</sub> species, although there are cases of insensitive stomatal responses [1,2,39]. Under a double-ambient growth [CO<sub>2</sub>], the reductions in stomatal conductance range from 14 to 50% for C<sub>4</sub> plants, compared to about 20% for C<sub>3</sub> species [2,35,39]. This stomatal reduction leads to a decrease in transpiration rate and eventually an improvement in WUE for plants grown at elevated [CO<sub>2</sub>]

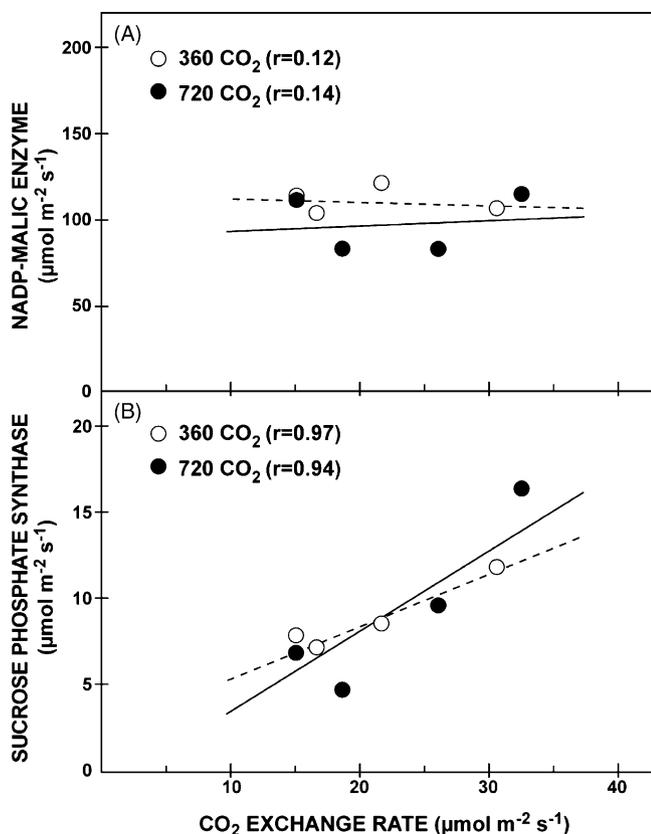


Fig. 8. CO<sub>2</sub> exchange rates (CER) vs. the activities of NADP-malic enzyme (A) and sucrose phosphate synthase (B) for outermost sections (near leaf tips) of leaf 7 of sugarcane plants grown at ambient (○, 360 μmol mol<sup>-1</sup>) and double-ambient (●, 720 μmol mol<sup>-1</sup>) CO<sub>2</sub>. Leaf CER and activities of the enzymes were determined at 7, 14, 32 and 48 days after leaf emergence. The solid line and broken line in each figure represent the best fitting lines for the double-ambient and ambient-CO<sub>2</sub> treatment, respectively, and the correlation coefficients (*r* values) are given in each figure.

[2,40]. In maize, mature leaves exposed to triple-ambient growth [CO<sub>2</sub>] (1100 μmol mol<sup>-1</sup>) had 71% lower stomatal conductance and 225% higher WUE than plants grown at 350 μmol CO<sub>2</sub> mol<sup>-1</sup> [14]. In the present study with sugarcane, stomatal conductance and transpiration (data not presented) were greatly reduced while leaf WUE was increased up to 52% for plants grown under double-ambient [CO<sub>2</sub>], when compared with their counterparts grown at ambient [CO<sub>2</sub>] (Fig. 1B). For C<sub>4</sub> plants, the reductions in stomatal conductance and subsequently leaf transpiration rate can lead to an increase in leaf temperature, a conservation of soil water resource and an improvement in shoot water status [34]. Such effects would eventually enhance leaf CER and leaf area production rate, leading to the increases in whole plant CO<sub>2</sub> assimilation rate and biomass for the elevated [CO<sub>2</sub>]-grown C<sub>4</sub> plants [34,35]. For sugarcane, an increase in total leaf area per plant at double-ambient growth [CO<sub>2</sub>] suggests that photosynthesis of the whole plant, and over-all carbon assimilation, would have been greater, and this may explain the over-all greater accumulation in total above-ground biomass for the elevated [CO<sub>2</sub>] plants (Table 1).

In addition to an increase in biomass accumulation, the improvement in WUE would be the greatest benefit C<sub>4</sub> plants may have under elevated growth [CO<sub>2</sub>], especially when soil

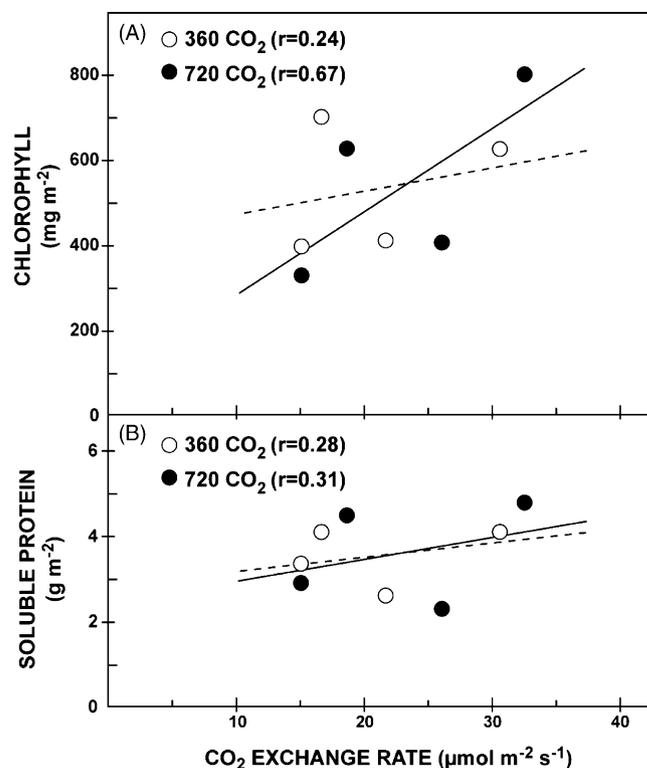


Fig. 9. CO<sub>2</sub> exchange rates (CER) vs. the concentrations of total chlorophyll (A) and soluble protein (B) for outermost sections (near leaf tips) of leaf 7 of sugarcane plants grown at ambient (○, 360 μmol mol<sup>-1</sup>) and double-ambient (●, 720 μmol mol<sup>-1</sup>) CO<sub>2</sub>. Leaf CER and concentrations of chlorophyll and protein were determined at 7, 14, 32 and 48 days after leaf emergence. The solid line and broken line in each figure represent the best fitting lines for the double-ambient and ambient-CO<sub>2</sub> treatment, respectively, and the correlation coefficients (*r* values) are given in each figure.

moisture is limiting [14,38]. As the atmospheric growth [CO<sub>2</sub>] is increased, the improvements in WUE are due to increased assimilation rate and decreased water loss, with the latter being more important under water-deficit situations [36]. Furthermore, as soil water becomes less available, the relative enhancement of photosynthesis and growth by elevated [CO<sub>2</sub>] tends to be greater, which can alleviate drought stress and delay its onset [41].

Growth of C<sub>4</sub> plants at elevated [CO<sub>2</sub>] can lead to an acclimation in the capacities of certain photosynthetic enzymes. In field-grown sorghum, activities of Rubisco and PEPC decrease at growth [CO<sub>2</sub>] of 200 μmol mol<sup>-1</sup> above the ambient level [32]. In maize grown at triple-ambient [CO<sub>2</sub>] and under high PPFD (2000 μmol m<sup>-2</sup> s<sup>-1</sup>), activities of a number of C<sub>3</sub> and C<sub>4</sub> cycle enzymes (Rubisco, PEPC, NADP-MDH, NADP-ME and PPDK) are down-regulated [14]. Sorghum plants grown at double-ambient [CO<sub>2</sub>] in controlled environment chambers (12-h photoperiod at 800 μmol m<sup>-2</sup> s<sup>-1</sup> PPFD) have a 49% reduction in leaf PEPC protein concentration, but no change in Rubisco protein content [29]. For sugarcane, Rubisco, PPDK and NADP-MDH activities of the elevated [CO<sub>2</sub>] plants were up-regulated at 14 DALE, while PEPC activities of both ambient- and elevated-CO<sub>2</sub> plants were similar during leaf development (Figs. 3 and 4). Variations in responses of the photosynthetic enzymes suggest that, among

C<sub>4</sub> plants, species-specific differences will be encountered as a result of future increases in atmospheric [CO<sub>2</sub>], in addition to differences in plant growth conditions and/or growth stages of the leaves.

In maize, activities of the enzymes of the sucrose and starch synthesis pathways (fructose 1,6-bisphosphatase and ADP-glucose pyrophosphorylase) are greater under triple-ambient growth [CO<sub>2</sub>], suggesting that the increased capacity to synthesize and utilize these photosynthetic end-products could contribute to the enhancement in leaf area and plant biomass at elevated [CO<sub>2</sub>] [14]. In sugarcane, activity of SPS and concentrations of sucrose and total soluble sugars were up-regulated at 14 DALE (Fig. 5B). This is consistent with the increases of SPS and sucrose in leaves of rice, a sucrose-accumulator C<sub>3</sub> plant, when exposed to long-term double-ambient growth [CO<sub>2</sub>] [42]. The basis for increases in SPS activity and sucrose concentration in sugarcane leaves by elevated [CO<sub>2</sub>] is still not well understood. However, since sucrose is the primary commodity being sought after in sugarcane, the implication of this for sugarcane production in the future is important, with the continued rise in atmospheric [CO<sub>2</sub>].

Exactly how an up- or down-regulation of certain C<sub>3</sub> and C<sub>4</sub> cycle enzymes by elevated [CO<sub>2</sub>] would regulate photosynthesis and growth of C<sub>4</sub> plants remains to be elucidated. However, a relatively clear picture on the role played by the individual enzymes in controlling the flux in C<sub>4</sub> photosynthesis has been established [43]. Through determination of the control coefficient for each enzyme of C<sub>4</sub> photosynthesis by measuring the CO<sub>2</sub> assimilation rate under saturating illumination at normal ambient [CO<sub>2</sub>], the bulk of control in C<sub>4</sub> photosynthesis is shown to lie primarily with the three enzymes Rubisco, PEPC and PPDK [43]. For sugarcane, leaf CER and activities of Rubisco, PEPC and PPDK are at highest levels when measured under full sunlight [44]. Data from the present study on sugarcane leaf CER and photosynthetic enzymes, determined on a clear midday at 14 DALE, were comparable to those measured at midday on uppermost fully expanded leaves of field-grown sugarcane plants [44]. Under normal ambient [CO<sub>2</sub>] and at full sunlight, sugarcane leaf photosynthesis may be regulated by PPDK activity, while CER may be limited by PEPC activity under low sunlight conditions [44]. For sugarcane in this study, there was a relatively good correlation of leaf photosynthetic rate with activity of Rubisco and PEPC, but not with PPDK, during the course of leaf ontogeny for both [CO<sub>2</sub>] treatments (Figs. 6 and 7A).

Data from the present study indicate that growth of sugarcane at double-ambient [CO<sub>2</sub>] could likely benefit through an enhancement in leaf CER, Chl and protein, and an up-regulation in the capacities of certain key photosynthetic enzymes and sucrose metabolism, during the early growth stages of the leaf. The up-regulation of these enzymes, together with the reductions in leaf stomatal conductance and transpiration and an improvement in leaf WUE under double-ambient [CO<sub>2</sub>], may lead to an enhancement in leaf area and total plant biomass accumulation and a greater sucrose production for the CO<sub>2</sub>-enriched sugarcane plants. The fact that

large treatment differences in leaf chlorophyll and protein concentrations and photosynthetic enzyme activities, which occurred at times when differences in leaf CER were small, is still an enigma and requires further investigation. Leaf CER and associated photosynthetic activities for the sugarcane plants of both ambient- and elevated-CO<sub>2</sub> treatments in a future study should be determined at more frequent intervals during the first few weeks following leaf emergence. Nevertheless, even a small, but consistent, percent stimulation in leaf CER as observed at various leaf growth stages in the current experiment (Fig. 1A) could also have a contribution to the enhancement in sugarcane plant biomass accumulation under elevated growth [CO<sub>2</sub>].

### Acknowledgments

We thank Ms. Joan Anderson for her skillful laboratory assistance, and Mr. Wayne Wynn and Mr. Andy Frenock for engineering support.

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