Stem juice production of the C₄ sugarcane (*Saccharum officinarum*) is enhanced by growth at double-ambient CO₂ and high temperature


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**Summary**

Two cultivars of sugarcane (*Saccharum officinarum* cv. CP73-1547 and CP88-1508) were grown for 3 months in paired-companion, temperature-gradient, sunlit greenhouses under daytime [CO₂] of 360 (ambient) and 720 (double ambient) μmol mol⁻¹ and at temperatures of 1.5 °C (near ambient) and 6.0 °C higher than outside ambient temperature. Leaf area and biomass, stem biomass and juice and CO₂ exchange rate (CER) and activities of ribulose bisphosphate carboxylase-oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPC) of fully developed leaves were measured at harvest. On a main stem basis, leaf area, leaf dry weight, stem dry weight and stem juice volume were increased by growth at doubled [CO₂] or high temperature. Such increases were even greater under combination of doubled [CO₂]/high temperature. Plants grown at doubled [CO₂]/high temperature combination averaged 50%, 26%, 84% and 124% greater in leaf area, leaf dry weight, stem dry weight and stem juice volume, respectively, compared with plants grown at ambient [CO₂]/near-ambient temperature combination. In addition, plants grown at doubled [CO₂]/high temperature combination were 2–3-fold higher in stem soluble solids than those at ambient [CO₂]/near-ambient temperature combination. Although midday CER of fully developed leaves was not affected by doubled [CO₂] or high temperature, plants grown at doubled [CO₂] were 41–43% less in leaf stomatal conductance and 69–79% greater in leaf water-use efficiency, compared with plants

**KEYWORDS**

Elevated atmospheric CO₂ and temperature; Leaf photosynthesis; Plant biomass; Stem juice; Sugarcane

**Abbreviations:** BRIX, percent of total soluble solids in stem juice extract; CER, CO₂ exchange rate; gₛ, stomatal conductance; PEPC, phosphoenolpyruvate carboxylase; PPFD, photosynthetic photon flux density; Rubisco, ribulose bisphosphate carboxylase-oxygenase; TGGs, temperature-gradient greenhouses; WUE, water-use efficiency.

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grown at ambient [CO₂]. Activity of PEPC was down-regulated 23–32% at doubled [CO₂], while high temperature did not have a significant impact on this enzyme. Activity of Rubisco was not affected by growth at doubled [CO₂], but was reduced 15–28% at high temperature. The increases in stem juice production and stem juice soluble solids concentration for sugarcane grown at doubled [CO₂] or high temperature, or at doubled [CO₂]/high temperature combination, were partially the outcome of an increase in whole plant leaf area. Such increase would enhance the ongoing and cumulative photosynthetic capability of the whole plant. The results indicate that a doubling of [CO₂] would benefit sugarcane production more than the anticipated 10–15% increase for a C₄ species.

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Introduction

The photosynthetic performance of many terrestrial plants is below their potential capability at current atmospheric CO₂ and O₂ levels. A rise in atmospheric CO₂ concentration ([CO₂]) by itself could stimulate photosynthesis and enhance productivity of important agricultural crops (Bowes, 1993; Kimball, 1993; Drake et al., 1997; Long et al., 2004). In C₃ photosynthesis, the CO₂ exchange rate (CER) in the leaf is a direct result of the activity of ribulose bisphosphate carboxylase-oxygenase (Rubisco), which is not saturated at current atmospheric [CO₂]. An increase in air [CO₂] availability results in increased leaf CER, because elevated [CO₂] inhibits the Rubisco oxygenase reaction and the subsequent loss of CO₂ through photorespiration (Bowes, 1996). In addition to atmospheric [CO₂], CER of C₃ plants is affected by air temperature, and this effect is also primarily exerted through Rubisco (Long, 1991). An increase in ambient temperature reduces the activation state of Rubisco (Kobza and Edwards, 1987; Holaday et al., 1992) and decreases both the specificity for CO₂ and the solubility of CO₂, relative to O₂ (Long, 1991). As growth temperature increases, the CO₂/O₂ ratio in solution is reduced and results in a decline in the Rubisco carboxylation/oxygenation ratio and an enhancement in photorespiration (Jordan and Ogren, 1984; Long, 1991; Bowes, 1993). Since the balance between the carboxylation and oxygenation reaction depends on the relative concentrations of CO₂ and O₂ at the Rubisco site, an increase in atmospheric [CO₂], and the concomitant inhibition of the Rubisco oxygenase reaction, should moderate the adverse impacts of high air temperature on C₃ photosynthesis (Long, 1991). It has been projected that atmospheric [CO₂], currently at about 385 μmol mol⁻¹, may surpass 700 μmol mol⁻¹ before the end of this century (Solomon et al., 2007). A rise in atmospheric [CO₂] and other greenhouse gases would cause an increase in the mean global air temperature by as much as 6 °C (Schneider, 2001; Solomon et al., 2007), and undoubtedly have a significant impact on photosynthesis and productivity of many plants. As a result, research on rising atmospheric [CO₂], and elevated [CO₂] interacting with high temperature or soil water deficit, has focused extensively in the last 30 years on C₃ plants, which represent more than 90% of terrestrial plant species (Bowes, 1993).

Plants of the C₄ photosynthetic category have evolved specific mechanisms to overcome the limitations of low atmospheric [CO₂], improve their photosynthetic efficiency and conserve their water use under hostile environmental conditions. By using the C₄ photosynthetic cycle to concentrate [CO₂] at the Rubisco site to levels manyfold higher than ambient [CO₂], C₄ plants are able to achieve a greater photosynthetic capacity than C₃ plants at the current atmospheric [CO₂], particularly at high growth temperatures (Matsuoka et al., 2001). Because of this unique CO₂-concentrating mechanism capability, photosynthesis of C₄ plants is practically near saturation at current atmospheric [CO₂], and therefore C₄ plants would not show significant growth responses to a rise in ambient [CO₂] (Bowes, 1993). Nevertheless, a positive growth response to elevated growth [CO₂] has been reported for a variety of C₄ plants, although to a smaller extent compared with the C₃ species (Kimball, 1993; Poorter et al., 1996; Ghannoum et al., 2000; Long et al., 2004). In spite of the fact that recent research progress has been made in characterizing the mechanisms of a limited number of C₄ species to increases in air levels of [CO₂] and temperature and changes in soil moisture status (Leakey et al., 2006; Vu et al., 2006; De Souza et al., 2008; Vu and Allen, 2009), the responses of C₄ crops to future rising [CO₂] and climate changes are still very variable and uncertain (Leakey et al., 2006; De Souza et al., 2008). Such uncertainties would limit predictions of future global climate change impacts.
on C₄-dominated agricultural and ecological systems. Although C₄ plants represent fewer than 4% of all angiosperm species, their ecological and economic significance is substantial (Brown et al., 2005). On a global basis, up to one-third of terrestrial productivity is provided by C₄ plants (Cerling et al., 1997; Ghannoum et al., 1997; Brown et al., 2005). In many tropical regions, the food source is primarily based on C₄ crops, among those maize, millet, sorghum and sugarcane are the most agriculturally important monocots in terms of production (Brown, 1999). Up to 75% of the world sugar production is provided by sugarcane (De Souza et al., 2008), with an estimation at about 150 million tonnes for the year 2005/06 (Glassop et al., 2007). In addition, the use of sugarcane as a source for biofuel production has been highly recognized (Goldenberg, 2007). Studies are therefore needed for advancing our understanding on growth and yield and the mechanisms underlying the photosynthesis and metabolism of this C₄ crop plant, as well as other economically important C₄ species, in response to the predicted changes in atmospheric [CO₂] and climate (Leakey et al., 2006).

In a previous study with sugarcane, we have demonstrated that elevated growth [CO₂] enhances leaf CER and up-regulates the capacity of certain key photosynthetic enzymes and sucrose metabolism in young developing leaves (Vu et al., 2006). In the present study, two cultivars of sugarcane with distinct characteristics in terms of tolerance to soil water availability were grown for 3 months in sunlit greenhouses under ambient and double-ambient [CO₂] and at temperatures up to 6.0 °C above outdoors. Leaf area and biomass, biomass and juice production of the main stem and CER and activity of the two C₄ photosynthesis carboxylating enzymes of fully developed leaves were determined at harvest. Our objective was to characterize the photosynthesis and growth performance of sugarcane under double-ambient [CO₂] and high temperature, and to test if growth of this C₄ monocot at doubled [CO₂] and high temperature enhanced the production of stem juice. Sugarcane has the characteristics of simplicity for sugar storage, as sucrose is the sugar which is synthesized in the leaf, translocated in the phloem and stored in the stem as soluble solids or BRIX (Moore, 1995). As a result, juice of the mature stems of sugarcane contains a very high concentration of sucrose (Moore, 1995; Lingle, 1999; Rae et al., 2005), which is extracted and purified during commercial sugar production. Understanding growth as well as photosynthesis of economically important C₄ crops in response to rises in atmospheric [CO₂] and temperature and variations in soil moisture availability is essential for predictions of how agricultural C₄ populations would perform under a future CO₂- and climate-changed world.

Materials and methods

Plant material and growth conditions

Two cultivars of sugarcane (Saccharum officinarum L.), CP73-1547 and CP88-1508, were grown in Gainesville, Florida (29°38’N and 82°22’W) in paired-companion, temperature-gradient greenhouses (TGGs). These two cultivars, with contrast characteristics in terms of tolerance to soil water availability (CP73-1547, a drought tolerant; CP88-1508, a flood tolerant), were developed through a cooperative program, based at Canal Point, Florida, of the USDA-ARS Sugarcane Production Research Station, the Florida Sugar Cane League, Inc. and the University of Florida Institute of Food and Agricultural Sciences, and have been widely grown for commercial production in South Florida. The TGGs, with semi-cylindrical arch-shape structures of 29.3-m long, 4.3-m wide and 2.2-m high at the ridgepole, were covered with a transparent greenhouse polyethylene plastic film which transmitted 90% of the solar photosynthetic photon flux density (PPFD) so that test plants received direct, natural solar irradiance. These TGGs provided technology to study a wide variety of agricultural plant species grown season-long under enriched [CO₂] and a range of temperatures (Fritschi et al., 1999; Vu et al., 2002, 2006; Newman et al., 2005; Allen and Vu, 2009). Each TGG was divided into a 3.6-m long entry section to stabilize incoming flow, four sequential experimental segments, each 5.5-m long, and a 1.8-m flow convergence zone before the air was expelled by a controlled-speed greenhouse ventilation fan. A computer-controlled, variable speed ventilation fan mounted at the south end of each TGG controlled air flow and regulated the temperature gradient continuously 24-h/d, which averaged from 1.5 °C above outside ambient temperature (Tₐ) (at the air-entry north end, segment 1) to 6.0 °C above Tₐ at the south end (segment 4). Dewpoint temperatures were measured in the outside ambient air and at the warmest end of the TGGs. Dewpoint temperature of the outside ambient air averaged 22 °C, and that of the warmest end of the TGGs averaged 24 °C at night and 30 °C during the day. The [CO₂] was maintained in one TGG at ambient concentration of about 360 µmol mol⁻¹ by flowing in air directly from
outside ambient, and at a doubled-ambient concentration of about 720 \( \mu \text{mol mol}^{-1} \) in the other. The \( \text{CO}_2 \) enrichment in the elevated-\( \text{CO}_2 \) TGG was implemented during daylight hours by injection of \( \text{CO}_2 \), provided from a supply tank outside the TGG, at 1.8 m into the air-entry segment of the TGG through a predilution system that provided cross-sectional uniform \( \text{CO}_2 \) concentrations. The [\( \text{CO}_2 \)] was measured continuously in segment 1 to control the rate of \( \text{CO}_2 \) injection for maintaining the set point [\( \text{CO}_2 \)], and once in every 20 min in segment 4. The structural characteristics, specific methods and quality of [\( \text{CO}_2 \)] and temperature controls in the TGGs were previously described in detail (Vu et al., 2002).

Sugarcane was propagated vegetatively in mid-January 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. Homogeneous germinated plants of each cultivar were transplanted in late March into the galvanized metal containers, 1.5-m long \( \times \) 0.6-m wide \( \times \) 0.6-m deep, containing organic soil. Eight containers, each having four germinated plants, two for each cultivar, were arranged in each of the two segments (1 and 4) of the two TGGs. Daytime [\( \text{CO}_2 \)] was maintained at about 360 \( \mu \text{mol mol}^{-1} \) (ambient) in one TGG, and 720 \( \mu \text{mol mol}^{-1} \) (doubled) in the other. The [\( \text{CO}_2 \)] values were set to allow comparing the current experiment to [\( \text{CO}_2 \)] treatments in experiments performed at this Gainesville location with other crop plants since the mid-1990s, when the value of “ambient” [\( \text{CO}_2 \)] was closer to 360 \( \mu \text{mol mol}^{-1} \). Soil moisture was checked daily, and additional irrigation applied as needed to ensure adequate soil moisture for plant growth. Fertilizers containing macro 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 (Obreza et al., 1998). During the months of January to June, minimum/maximum temperatures outside the TGGs were 7.1/20.5, 9.7/23.2, 14.2/27.4, 12.0/25.9, 16.4/29.7 and 19.7/30.7 °C, respectively. In late June, most sugarcane plants had their main shoot tips touching the TGG’s plastic roof, which created greater crowding of shoot space and would damage the roof if plants were kept longer for further growth. Experiments were therefore terminated at this growth stage, and leaf area and biomass, main stem biomass and juice, leaf photosynthesis and activities of the photosynthetic carboxylating enzymes were evaluated.

**Measurements of leaf area, plant biomass and stem juice**

From segments 1 and 4 of each TGG, four plants were sequentially harvested for each cultivar. Leaves and stems were separated, and total leaf area was determined for each individual plant. An electric crusher was used to extract the stem juice. Only juice volume for each major main stem was measured. The extracted juice was then filtered through six layers of cheesecloth, and the percentage by weight of total soluble solids in the extracted juice (BRIX) was measured, using both a hydrometer and a handheld refractometer. Leaves and stems were then oven-dried at 70 °C, and their dry weights were determined.

**Leaf gas exchange measurements**

Leaf CER and conductance (\( g_s \)) of uppermost fully expanded attached leaf blades were determined in situ for the two cultivars of segments 1 and 4 of each TGG. Measurements were made at midday, between 1000 and 1400 EDT (1400–1800 \( \mu \text{mol mol}^{-1} \) \text{m}^{-2} \text{s}^{-1} \text{PPFD}), on outermost sections (near leaf tips), using a LI 6200 Photosynthesis System and LI 6000-12 (1-dm\(^3\) volume) cuvette (LI-COR, Lincoln, NE), as previously reported (Vu et al., 2006). The duration of each measurement was typically 30–45 s, and increases in leaf temperature during such short measurement periods were less than 3% compared with air temperature. Leaf CER and \( g_s \) were expressed on a leaf area basis, and the water-use efficiency (WUE) was calculated as the ratio of CER to \( g_s \).

**Leaf sampling and assay of the enzymes**

Following gas exchange measurements, five outermost sections (near leaf tips) were detached from the leaf blades of plants for both cultivars of segments 1 and 4 of each TGG, and were immediately immersed in liquid \( \text{N}_2 \). Sampled leaves were pooled by treatment, ground to a fine powder in liquid \( \text{N}_2 \), and continuously stored in liquid \( \text{N}_2 \) until analysis. Leaf fresh weight and area were also determined for a subset of plants at the same time of leaf sampling for biochemical analysis.

From the liquid-\( \text{N}_2 \) frozen leaf powder, Rubisco and phosphoenolpyruvate carboxylase (PEPC) were extracted and activities were assayed. About 90 mg of the frozen leaf powder was transferred to a precooled Ten-Broeck homogenizer and ground at 2 °C in 1.8 mL of extraction medium which consisted of 100 mM Bicine-NaOH (pH 8.0), 10 mM MgCl\(_2\), 0.1 mM...
EDTA-Na₂, 5 mM DTT, 10 mM isococarbote, 2% (w/v) PVP-40 and 0.1% (v/v) Triton X-100. The homogenate was micro-centrifuged at 12,000g for 45 s at 2 °C, and the supernatant was immediately assayed for enzyme activities. Both Rubisco and PEPC were assayed spectrophotometrically at 340 nm in a total volume of 0.5 mL at 25 °C, in triplicate. For Rubisco, 0.2 mL aliquot of the supernatant was first incubated with 0.01 mL of 500 mM MgCl₂ and 0.01 mL of 200 mM NaHCO₃. After a 5-min activation period, activity of Rubisco was assayed as described by Sharkey et al. (1991). The reaction mixture contained 100 mM Bicine-NaOH (pH 8.0), 20 mM MgCl₂, 1 mM EDTA-Na₂, 20 mM NaCl, 10 mM NaHCO₃, 5 mM DTT, 2.5 mM ATP, 5 mM phosphocreatine, 5 units of creatine phosphokinase, 5 units each of PGA kinase and GAP dehydrogenase, 0.2 mM NADH, and 0.6 mM RuBP. After a steady baseline absorbance was established, the reaction was initiated with 0.01 mL of the activated extract. The linear decrease in absorbance resulting from oxidation of NADH was recorded over a period of 150 s.

Activity of PEPC was assayed using a modification of the procedure of Ashton et al. (1990). The reaction mixture contained 100 mM Bicine-NaOH (pH 8.0), 10 mM MgCl₂, 0.1 mM EDTA-Na₂, 10 mM NaHCO₃, 5 mM DTT, 2.5 units of MDH and 0.2 mM NADH. After addition of 0.01 mL of enzyme extract, a steady base was established and the reaction was initiated by adding PEP to a final concentration of 5 mM. The linear decrease in absorbance was recorded over a period of 150 s.

Statistical analysis

A two-way analysis of variance was conducted for each cultivar to test for the [CO₂] × temperature and [CO₂] × temperature interaction effects. Comparisons between means of different treatments for each cultivar were performed using the Duncan multiple range test (Gomez and Gomez, 1984).

Results

Leaf area

Total leaf area per main stem was enhanced in the two cultivars of sugarcane grown at double-ambient [CO₂] and high temperature (segment 4, Tₐ + 6.0 °C) (Figure 1). Doubled [CO₂] enhanced leaf area up to 31% under growth at near-ambient temperature (segment 1, Tₐ + 1.5 °C) and 36% under growth at high temperature. Under high temperature, leaf area of the ambient [CO₂] plants increased up to 25%, and that of the doubled [CO₂] plants was enhanced up to 28%. The increases were even greater for plants grown under combination of doubled [CO₂]/high temperature. Leaf area of plants grown at doubled [CO₂]/high temperature, when compared with plants grown at ambient [CO₂]/near-ambient temperature, was enhanced by 50% on average.

Stem juice

Sugarcane juice production per main stem was enhanced substantially by growth at doubled [CO₂] and high temperature (Figure 2). Plants grown at doubled [CO₂]/high temperature combination, compared with plants grown at ambient [CO₂]/near-ambient temperature combination, had stem juice increased up to 165%. Growth at high temperature alone had stem juice increased up to 94% for the ambient [CO₂] plants and 48% for the doubled [CO₂] plants. The increases in stem juice for plants grown at doubled [CO₂], when compared with plants grown at ambient [CO₂], were up to 83% under near-ambient temperature and 91% under high temperature.
Leaf and stem biomass

The average increases in leaf dry weight for plants of both cultivars grown at doubled [CO₂]/high temperature were 25%, compared with plants grown at ambient [CO₂]/near-ambient temperature (Figure 3A). Although the enhancements in leaf biomass by doubled [CO₂] or high temperature alone were less evident compared with the doubled [CO₂]/high temperature combination, they were still noticeable.

Plants grown at doubled [CO₂]/high temperature averaged 84% higher in stem dry weight, compared with plants grown at ambient [CO₂]/near-ambient temperature (Figure 3B). Ambient [CO₂] plants grown at high temperature, compared with counterpart plants grown at near-ambient temperature, had up to 63% more in stem dry weight. Similarly, doubled [CO₂] plants grown at high temperature averaged 49% greater in stem dry weight, compared with doubled [CO₂] plants grown at near-ambient temperature. Doubled [CO₂] plants grown at near-ambient temperature, compared with ambient [CO₂] plants grown at ambient temperature, averaged 24% more in stem dry weight.

Total whole plant dry weight per main stem, which is the sum of the leaf and stem dry weights, reflects a similar trend as shown in Figures 3A and B for the two sugarcane cultivars grown at two [CO₂] and two temperatures. Therefore, patterns for total plant dry weight will not be presented here. In general, plants grown at doubled [CO₂]/high temperature were 64% greater in total dry weight than those at ambient [CO₂]/near-ambient temperature, whereas plants grown at doubled [CO₂]/near-ambient temperature, compared with those at ambient [CO₂]/near-ambient temperature, had total dry weight increased by 16%. With respect to growth temperature, ambient [CO₂] plants grown at high temperature were 31% greater in total dry weight than ambient [CO₂] plants grown at near-ambient temperature. Similarly, doubled [CO₂] plants grown at high temperature were 42% higher...
### Table 1. Leaf CO2 exchange rate (CER), conductance (g), water use efficiency (WUE), activity of PEP carboxylase (PEPC) and Rubisco, stem juice BRIX and stem-soluble solids of the two cultivars of sugarcane, CP73-1547 and CP88-1508, grown under ambient and double-ambient [CO2] and at temperatures (T) of 1.5 and 6.0 °C above ambient temperature (Tₐ).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>360 μmol mol⁻¹ CO₂</th>
<th>720 μmol mol⁻¹ CO₂</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tₐ+1.5 °C</td>
<td>Tₐ+6.0 °C</td>
<td>Tₐ+1.5 °C</td>
</tr>
<tr>
<td><strong>CP73-1547</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CER (μmol CO₂ m⁻² s⁻¹)</td>
<td>36.5 (5.2) a</td>
<td>34.8 (3.7) a</td>
<td>38.0 (4.4) a</td>
</tr>
<tr>
<td>g (mmol H₂O m⁻² s⁻¹)</td>
<td>352 (49) a</td>
<td>314 (43) a</td>
<td>187 (34) b</td>
</tr>
<tr>
<td>WUE (mol CO₂ mol⁻¹ H₂O)</td>
<td>104 (9) b</td>
<td>111 (12) b</td>
<td>203 (21) a</td>
</tr>
<tr>
<td>PEPC (μmol CO₂ m⁻² s⁻¹)</td>
<td>298 (21) a</td>
<td>300 (20) a</td>
<td>214 (8) b</td>
</tr>
<tr>
<td>Rubisco (μmol CO₂ m⁻² s⁻¹)</td>
<td>39 (2) a</td>
<td>34 (1) a</td>
<td>38 (3) a</td>
</tr>
<tr>
<td>Stem juice BRIX (%) (w/v)</td>
<td>8.6 (0.1) a</td>
<td>9.7 (0.9) a</td>
<td>7.8 (0.4) a</td>
</tr>
<tr>
<td>Soluble solids (g main stem⁻¹)</td>
<td>8.2 (0.3) d</td>
<td>17.9 (1.3) b</td>
<td>13.6 (0.7) c</td>
</tr>
<tr>
<td><strong>CP88-1508</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CER (μmol CO₂ m⁻² s⁻¹)</td>
<td>36.8 (4.5) a</td>
<td>34.5 (6.0) a</td>
<td>37.2 (3.2) a</td>
</tr>
<tr>
<td>g (mmol H₂O m⁻² s⁻¹)</td>
<td>330 (41) a</td>
<td>330 (40) a</td>
<td>178 (33) b</td>
</tr>
<tr>
<td>WUE (mol CO₂ mol⁻¹ H₂O)</td>
<td>112 (12) b</td>
<td>115 (17) b</td>
<td>209 (22) a</td>
</tr>
<tr>
<td>PEPC (μmol CO₂ m⁻² s⁻¹)</td>
<td>268 (14) a</td>
<td>301 (19) a</td>
<td>222 (12) b</td>
</tr>
<tr>
<td>Rubisco (μmol CO₂ m⁻² s⁻¹)</td>
<td>42 (3) a</td>
<td>33 (2) b</td>
<td>39 (3) ab</td>
</tr>
<tr>
<td>Stem juice BRIX (%) (w/v)</td>
<td>7.6 (1.4) a</td>
<td>8.3 (1.3) a</td>
<td>7.0 (0.3) a</td>
</tr>
<tr>
<td>Soluble solids (g main stem⁻¹)</td>
<td>6.9 (1.2) b</td>
<td>7.2 (1.1) b</td>
<td>7.8 (0.2) a</td>
</tr>
</tbody>
</table>

Values are the means and S.E (parentheses) of eight determinations for CER, g, and WUE, four for BRIX and soluble solids, and three for PEPC and Rubisco. Values within rows with the same letter indicate no significant difference (p<0.05) in a Duncan multiple range test.

In total dry weight than doubled [CO₂] plants grown at near-ambient temperature.

### Discussion

Sugarcane plants grown for 3 months at doubled [CO₂]/high temperature combination accumulated more leaf area and leaf and stem biomass than counterpart plants grown at ambient [CO₂]/near-ambient temperature combination (Figures 1 and 3). In addition, plants grown at doubled [CO₂] or high temperature, or at doubled [CO₂]/high temperature combination, had greater stem juice production than plants grown at ambient [CO₂]/near-ambient temperature combination (Figure 2).

Such enhancements in leaf area, biomass and stem juice were generally greater for plants grown at doubled [CO₂]/high temperature combination than for those grown either at elevated [CO₂] or high temperature alone. A recent study by De Souza et al. (2008) showed that sugarcane plants grown for 50 weeks at double-ambient [CO₂] accumulate 25% and 60% more in leaf and stem dry biomass than plants grown at ambient [CO₂].

In C₃ plants, elevated [CO₂] enhances leaf CER and growth by inhibiting photorespiration and increasing carboxylation by Rubisco, in addition to a reduction in leaf conductance (through partial closure of the stomata) and an improvement in leaf WUE (Drake et al., 1997; Leakey et al., 2006). For C₄ plants, however, the responses to elevated [CO₂] are variable and uncertain (Leakey et al., 2006; De Souza et al., 2008). Wild C₄ grasses grown at elevated [CO₂] are able to reduce leaf conductance, enhance leaf CER and stimulate plant biomass under both normal and stressed situations (Wand et al., 1999). In contrast, soil moisture and nutrient levels in the root zone play an important role in controlling the responses of maize and sorghum to elevated [CO₂] (Ottman et al., 2001; Cousins and Bloom, 2003; Leakey et al., 2006). For both maize and sorghum, photosynthesis and yield are not affected by elevated growth [CO₂] under well-watered conditions (Ottman et al., 2001; Leakey et al., 2006). In addition, the availability of soil nutrients influences photosynthesis and growth responses of maize and several other C₄ grasses to elevated [CO₂] (Cousins and Bloom, 2003; Tang et al., 2006). In sugarcane, CER of fully expanded leaves is not stimulated under drought-imposed conditions (Vu and Allen, 2009). De Souza et al. (2008) measured midday CER for the first leaf of well-watered sugarcane grown for 50 weeks in open-top chambers at both ambient and double-ambient [CO₂]. They showed an average increase in
CER of 35% by doubled [CO2] between the 6th and 22nd week, with a maximum enhancement of ~60% occurring at the 13th week. In addition, leaf gs decreases on average by 37%, while WUE is 62% greater under elevated [CO2] than that of the ambient-CO2 plants, with the highest stimulation (20%) occurring at 7 days after leaf emergence. The elevated-CO2 sugarcane plants also have up to 51% lower leaf gs, and 26–52% greater leaf WUE than ambient-CO2 plants during periods of leaf growth and development (Vu et al., 2006). It is important to point out that the cultivar, the experimental control and plant growth as well as the growth stages of the leaves used for CER determinations as reported by De Souza et al. (2008) were different from the cultivars and growth conditions used in our experiments. As a matter of fact, rising global [CO2] might not have the same effects on plants in all environments, and precaution therefore should be taken in extending comparisons or predictions of research findings of different plants or plant cultivars for various growth conditions (Allen and Vu, 2009). Nevertheless, the results from our previous study (Vu et al., 2006) and those as reported by De Souza et al. (2008) with different cultivars and at dissimilar growth conditions show that there is a stimulation in leaf CER and an improvement in leaf WUE for sugarcane grown at elevated [CO2], with the enhancement in CER occurring more evidently during the young stages of leaf/plant growth and development. An improvement in WUE as a result of long-term exposure to elevated [CO2] would likely be more important than the increase in net CO2 uptake per se in terms of plant growth and final yield (Chaves and Pereira, 1992).

In this study, leaf CER of ambient and doubled-ambient [CO2] sugarcane plants grown at high temperature (6°C above ambient) were slightly less, even though not significant, compared with those of their counterparts grown at near-ambient temperature (Table 1). Such small decreases in CER at high temperature might be the result of the increases in vapor pressure deficit, which was not quantified for the [CO2] and temperature treatments of this study. As the vapor pressure deficit might have an effect on leaf photosynthesis and plant growth responses in studies with elevated [CO2] and high temperature, control and measurement of the vapor pressure deficit should be an essential part of environmental controls in all future controlled environment research, especially as related to global climate change (Allen and Vu, 2009).

In young developing leaves of sugarcane, there is an up-regulation of the C4 photosynthetic enzymes, including PEPC and Rubisco, at elevated [CO2]
As leaves reach maturity, growth of sugarcane at doubled [CO₂] resulted in decreased activities of PEPC and Rubisco (Table 1). Such decreases in enzyme activities by doubled [CO₂] in mature sugarcane leaves have been also reported (Vu and Allen, 2009). High growth temperature, however, did not affect activity of PEPC but decreased that of Rubisco in sugarcane. In the C₄ pasture grasses Panicum coloratum and Centurus ciliaris and C₄ dicot Flaveria bidentis, there are no differences in PEPC activity in growth temperatures of 25 and 35 °C, while the amount of Rubisco catalytic sites per unit leaf area is lower at high growth temperature (Dwyer et al., 2007).

In sugarcane, the stem is made up of internodes at various stages of development (Moore, 1995; Lingle, 1999), and metabolic activities in the stem storage tissues are not similar from one internode to another, as sucrose concentration starts to increase in the internodes as elongation ceases (Rae et al., 2005; Glassop et al., 2007). The sugarcane stem juice BRIX, which consists of high percent of water-soluble sugars (primarily sucrose), provides a good estimate of the sugar content for stem juice (Moore, 1995). Immature expanding internodes at the top of the plant (internode 2) have relatively small BRIX accumulation rate (6–10%) and low concentration of sucrose (~100 mM), while the more developed and mature internodes located lower down the plant (internodes 10–40) have BRIX increasing by 2–3-fold and stem sucrose accumulating up to 650 mM (Welbaum and Meinzer, 1990; Moore, 1995; Rae et al., 2005). In the present study, BRIX of the whole stem juice extract averaged 9.1 for cultivar CP73-1547 and 7.8 for cultivar CP88-1508 (Table 1). Since our sugarcane plants were only 3-month old and main stems had about 10 internodes at time of harvest, they were still in their expanding stage, and stem juice BRIX values were therefore low. However, these BRIX values were well in the range as reported by Moore (1995) for young sugarcane internodes. In sugarcane, sucrose accounts for ~70% of the stem juice soluble solids in the lower internodes and can exceed 90% in fully mature internodes (Moore, 1995), and there is variability among sugarcane cultivars in how much sucrose is stored in the stem internodes (Lingle, 1999). For the two sugarcane cultivars used in this study, values of stem juice BRIX and soluble solids per main stem were generally higher for CP73-1547 than for CP88-1508. In addition, enhancements in stem juice BRIX and soluble solids by growth especially at doubled [CO₂]/high temperature combination were greater for cultivar CP73-1547 (Table 1). This would indicate that cultivar-specific difference with respect to sucrose productivity will be encountered for the C₄ sugarcane as a result of future increases in global [CO₂] and temperature.

In the present study, an increase in whole plant leaf area might partially explain an improvement in plant biomass and a stimulation in total juice production and soluble solids per main stem for sugarcane grown at doubled [CO₂] or high temperature, or under doubled [CO₂]/high temperature combination. An increase in leaf area per se would enhance the ongoing and cumulative photosynthetic capability of the whole plant. In addition, an improvement in leaf WUE under doubled [CO₂] and high temperature, as observed at various growth stages of the leaf (Vu et al., 2006; Vu and Allen, 2009), would also contribute to the increases in sugarcane biomass accumulation, stem juice production and stem sugars. Differences in genotypes, however, should be also taken into consideration. Thus, in the absence of other climatic stresses, sugarcane grown under predicted rising atmospheric [CO₂] and temperature in the future may use less water, utilize water more efficiently and perform better in sucrose production. With the worldwide continued increase in demand for sugarcane as a source of food and biofuel production, the improvements in stem sucrose and biomass through classical breeding and/or new biotechnology should be carried out with high priority. In addition, studies to identify the cultivars with high efficiency in water use and stem sucrose production under future changes in [CO₂] and climate are of great importance and should be initiated and explored.

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References


