

Measuring and modelling CO₂ effects on sugarcane



C.J. Stokes^{a,*}, N.G. Inman-Bamber^{b,c}, Y.L. Everingham^{c,d}, J. Sexton^{c,d}

^a CSIRO Land and Water, ATSIP JCU Campus, James Cook University, ATSIP Building 145, James Cook Drive, Townsville, 4811, Queensland, Australia

^b CSIRO Agriculture, ATSIP JCU Campus, James Cook University, ATSIP Building 145, James Cook Drive, Townsville, 4811, Queensland, Australia

^c College of Science, Technology and Engineering, School of Engineering and Physical Science, James Cook University, James Cook Drive, Townsville, 4811, Queensland, Australia

^d Centre for Tropical Environmental & Sustainability Science, College of Science, Technology and Engineering, School of Engineering and Physical Science, James Cook University, James Cook Drive, Townsville, 4811, Queensland, Australia

ARTICLE INFO

Article history:

Received 24 November 2014

Received in revised form

23 November 2015

Accepted 25 November 2015

Available online 7 January 2016

Keywords:

Sugarcane

CO₂

Transpiration

Glasshouse

Adaptation

ABSTRACT

In order to fully capture the benefits of rising CO₂ in adapting agriculture to climate change, we first need to understand how CO₂ affects crop growth. Several recent studies reported unexpected increases in sugarcane (C₄) yields under elevated CO₂, but it is difficult to distinguish direct leaf-level effects of rising CO₂ on photosynthesis from indirect water-related responses. A simulation model of CO₂ effects, based purely on changes in stomatal conductance (indirect mechanism), showed transpiration was reduced by 30% (initially) to 10% (closed canopy) and yield increased by 3% even in a well-irrigated crop. The model incorporated the results of a field experiment, and a glasshouse experiment designed to disentangle the mechanisms of CO₂ response: whole-plant transpiration and stomatal conductance were both 28% lower for plants growing with high-frequency demand-based watering at 720 vs 390 ppm CO₂, but there was no increase in biomass, indicating that indirect mechanisms dominate CO₂ responses in sugarcane.

© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Some of the most important sources of human and animal food are derived from a small number of the world's plant species that possess the CO₂ concentration mechanism that involve four carbon compounds. These C₄ species include maize, sorghum and sugarcane. Photosynthesis occurs in two types of tissue in C₄ plants, the mesophyll and the bundle sheath while only the mesophyll is involved in the case of C₃ plants (Matsuoka et al., 2001). This allows C₄ plants to reach maximum photosynthesis rates at current levels of ambient CO₂ (Ghannoum et al., 2000). Reported responses to elevated CO₂ concentrations of increased photosynthesis and consequent biomass accumulation in well watered C₄ plants (e.g., de Souza et al., 2008), have therefore been difficult to explain. In order to accurately model the impacts of climate change on crops and to fully capture the benefits of rising CO₂ in adapting agriculture to climate change, it is first necessary that we properly understand the processes by which CO₂ affects crop growth.

Even in well watered C₄ plants, elevated CO₂ and consequently reduced stomatal conductance can lead to enhanced leaf growth and photosynthesis through mitigating the effects of transient water stress (Seneweera et al., 1998). In an open top chamber experiment where sugarcane was exposed to twice normal CO₂, stomatal conductance was reduced by 37% and transpiration reduced by 32% while photosynthesis and biomass yield increased by 30% and 40% respectively (de Souza et al., 2008). de Souza et al. (2008) also found difficulty in explaining this result and suggested that even though plants were irrigated when soil water was at a low tension of 20 kPa, plants in normal CO₂ must have experienced transient water stress that was alleviated in the treatments under elevated CO₂. One would need to provide a different water regime for plants growing in high levels of CO₂ for them to experience the same degree of water stress to plants growing in normal CO₂ levels. Vu and Allen (2009) reported a similar reduction in conductance (34%) and a smaller reduction in transpiration (25%) when well watered sugarcane plants were provided with 'twice normal' CO₂ (720 ppm) in glasshouse experiments. Ghannoum et al. (2000) listed reports which indicate that assimilation (A) and biomass accumulation in well watered C₄ plants both increase under elevated CO₂ and other reports where A responded but not growth and yet others where growth responded but not A. These

* Corresponding author.

E-mail addresses: chris.stokes@csiro.au (C.J. Stokes), geoff.inmanbamber@gmail.com (N.G. Inman-Bamber), yvette.everingham@jcu.edu.au (Y.L. Everingham), justin.sexton@my.jcu.edu.au (J. Sexton).

conflicting results probably reflect the difficulty of inferring growth responses from short-term measurements of A using small segments of young leaves (Ghannoum et al., 2000), as was the case in the studies by de Souza et al. (2008) and Vu and Allen (2009). Ghannoum et al. (2000) argued that indirect effects are dominant in the response of C_4 plants to elevated CO_2 . Ghannoum et al. (2003) provided further evidence that even under water stress, elevated CO_2 does not directly enhance A , and any enhancement of A is most likely due to non-stomatal means. To date little work has been conducted on the effects of CO_2 on sugarcane and the contrasting reports among the work that has been done (de Souza et al., 2008; Vu and Allen, 2009) suggest that further investigation on the nature and mechanisms of these responses is warranted.

Tubiello et al. (2007) reviewed the literature on crop CO_2 experiments and concluded there was broad agreement between different techniques (across enclosed, semi-enclosed and unenclosed environments) for estimates of the impact of elevated CO_2 on yield, once differences in CO_2 treatment levels were taken into account. They also found that the results from most crop model simulations were consistent with the results from field-based CO_2 experiments.

Two sugarcane modelling platforms are available internationally for sugarcane, Canegro in the DSSAT platform (Inman-Bamber et al., 1993; Kiker et al., 2002; Singels et al., 2008) and the 'Sugar' module in the APSIM platform (Keating et al., 1999; Holzworth et al., 2014). In previous applications of these models to climate change, approaches to representing CO_2 effects on sugarcane have varied (Park et al., 2007; Webster et al., 2009; Marin et al., 2013; Knox et al., 2010; Biggs et al., 2013; Singels et al., 2014; Marin et al., 2015). Webster et al. (2009) assumed that intrinsic transpiration efficiency (TE) as defined by Sinclair, 2012 and Keating et al. (1999) would increase by 8% for every 100 ppm increase in CO_2 when estimating sugarcane yields for future climates in Australia, using the APSIM-Sugar model. This increase in TE is less than was assumed for wheat (10.6%) by Asseng et al. (2004) who used the APSIM module for wheat. However the response for wheat included benefits to TE from increased internal CO_2 as external CO_2 levels rise (Asseng et al., 2004) which may not be the case for sugarcane and other C_4 plants. Webster et al. (2009) also assumed that radiation use efficiency (RUE) would increase by 1.43% for every 100 ppm increase in CO_2 concentration. The assumptions about TE and RUE for sugarcane came from an internal report by Park et al. (2007). Biggs et al. (2013) used the same model and assumptions to predict yield and off-site impacts in future climates, for one sugarcane region. TE responses to twice normal CO_2 measured in small cuvettes, supported the Webster et al. (2009) assumption about TE in one case (Vu and Allen, 2009) but not in another where TE increased 62% in twice normal CO_2 (de Souza et al., 2008). The modest increase in RUE assumed by Webster et al. (2009) would not account for the 40% increase in biomass for well watered plants in twice normal CO_2 (de Souza et al., 2008). None of these modelling studies appeared to use experimental evidence for assumptions about the effects of CO_2 on sugarcane yield building processes.

Controlled environment experiments allow the fine level of manipulative control required to separate proximate leaf-level CO_2 responses from those mediated by whole-plant-soil hydrological feedbacks. We describe an experimental approach that is well suited to testing and measuring the mechanisms that should be represented in crop models. We also describe a field experiment used to measure transpiration and provide a baseline for crop simulations under current CO_2 levels. We then developed a model (from existing ones) that could make use of the results obtained in the glasshouse to predict what will happen in a field of sugarcane

subjected to elevated CO_2 .

This paper makes advancements, firstly in developing experimental techniques to decouple direct and indirect effects of enriched CO_2 on plants and secondly in improving ways to simulate the effects of CO_2 enrichment on sugarcane growth and water use.

2. Materials and methods

2.1. Glasshouse system

For the glasshouse pot experiments we developed a control system that could maintain soil water content within a narrow range to limit CO_2 responses mediated by feedbacks on soil moisture while simultaneously measuring the amount of water used. This was accomplished by using a sensor array linked to a control system that could independently control and deliver exact volumes of water to each pot (Fig. 1). Each pot was fitted with a volumetric soil moisture sensor (CS616, Campbell Scientific, Utah) that measured soil moisture every 10 min. If the soil moisture for a particular pot fell below its target treatment level, then a solenoid would open to deliver an aliquot of water from a precisely-machined reservoir (204 ml) above the pot. A data logger was programmed to automatically calibrate each sensor for the most recent three water deliveries (based on the increase in soil moisture in response to each addition of the known volume of water) so that water use could be interpolated between triggered water deliveries. A horizontal tube with lateral perforations, orientated perpendicularly to the moisture sensors, was used to spread the delivered water evenly in the pots, assisted further by a thin (2 cm) layer of sand between the tube and the soil surface. A 5 cm layer of plastic beads was placed on top of this to limit evaporation.

For the CO_2 treatments we used two large (approximately 6 m × 9 m by 7 m tall) controlled environment chambers, part of a new facility based on the design described in Inman-Bamber et al. (2008). The chambers included control systems for regulating temperature, humidity and CO_2 levels, with large air handlers to ensure even mixing. Two precautions were taken to prevent the build-up of plant-active contaminants from the CO_2 supply in chambers such as ethylene (Morison and Gifford, 1984a): 1) the system used a continuous flow of air through the chambers, with CO_2 injected into the incoming air stream (diluted in two-stage mixing to within 10 ppm of chamber levels before entering the chamber to eliminate CO_2 gradients); and 2) the source of gas used was produced by separation of atmospheric air, and therefore low in plant active impurities to begin with.

Air temperature and relative humidity (RH) were measured every minute with shielded sensors (HMP45a Vaisala Pty Ltd Melbourne, VIC) placed at the level of the leaves. Solar radiation (350–2500 nm) was measured above the plant canopy at a height of 6 m in each chamber with four 1-m long tube solarimeters (Delta-T Devices Ltd, Cambridge, UK).

The experiment was a factorial design of two CO_2 treatments by two sugarcane varieties by four harvest dates, replicated four times (with harvest dates and replicates arranged in a 4 × 4 Latin Square), giving a total of 64 pots. For the CO_2 treatments, one growth chamber was supplied with ambient air (approximately 390 ppm CO_2), while the other was elevated to approximately 720 ppm. The two varieties that were used were KQ228^A and Q208^A, two of the mostly widely grown commercial varieties in northern Queensland, Australia. The watering control system was set to maintain the soil in each pot at 90% of field capacity (watering trigger threshold). The four harvest dates are explained below.

One-eyed setts were germinated and then transplanted, three per pot, into pots (520 mm tall and 380 mm in diameter) containing 25 L of a premium potting mix. Plants were allowed to establish for

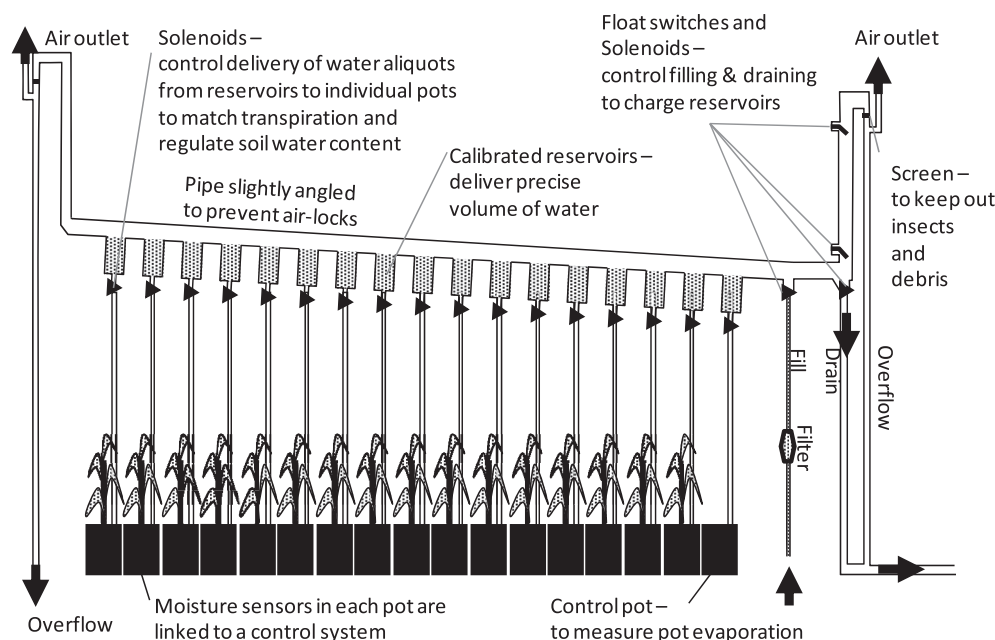


Fig. 1. Watering system used to precisely control soil water content and measure plant water use.

2 months at field capacity. During this period, pots were watered to just above field capacity each afternoon, and excess water was allowed to drain through small holes at the bottom of each pot. The water content after drainage was regarded as field capacity and was determined separately for each moisture sensor–pot combination. Watering and CO₂ treatments were then initiated and the starting mass of plants was determined from destructive harvesting of 10 extra plants of each variety. In each chamber four control pots were set up that were identical to experimental pots except that they contained no plants. These were used to measure pot evaporation (subtracted from pot water use in calculating transpiration).

Plants were harvested in batches at approximately 8-week intervals following the initiation of treatments. Harvested plants were subsampled and separated by plant part into green leaf, dead leaf, sheath and stalk to obtain dry masses following the procedures described in [Inman-Bamber et al. \(2008\)](#). Subsamples of green leaf were processed through a leaf area meter, and the height and number of internodes were measured for stalks. Water use for each pot was calculated for the final seven days before harvest, to calculate the water use per unit green leaf area for each harvest date.

Shortly before the final harvest in November 2011, gas exchange measurements were taken on the youngest fully expanded leaf (leaf #1) of 24 plants in each glasshouse, using a portable photosynthesis apparatus (Li-6400, Li-Cor Inc., Lincoln, NE, USA). A 6 cm² section of the leaf was enclosed in the cuvette and exposed to 2000 $\mu\text{mol}/\text{m}^2/\text{s}$ photosynthetically active radiation and to 375 ppm CO₂. Readings were later repeated on the same leaves using the same settings but this time with CO₂ set to 720 ppm.

Because of teething problems with the first experiment, we briefly note some clarifying results from a later experiment that addressed these issues. This later experiment included eight replicates of each of the same clones (Q208 and KQ228) and CO₂ treatment combinations as before. The experiment ran for a shorter period (12 weeks) so the plants were small enough to swap pots and CO₂ treatments between chambers half way through the trial. The average water use for each pot was calculated for the four days before the changeover and the four days after the changeover. An

analysis of variance was then used to test whether there were any effects of chambers, besides those associated with CO₂ treatments, on plant water use. At the end of the 12-week experiment, pots were harvested as before, and results were analysed to test for any biomass differences between treatments.

2.2. Field experiment

A Bowen ratio energy balance (BREB) system was set up in a 10.3 ha commercial block of sugarcane (cv. Q127, first ratoon) at Kalamia estate (19.6 °S, 147.4 °E), near Ayr in the Burdekin district, north-east Australia. The details and results of the BREB system were provided by [Inman-Bamber and McGlinchey \(2003\)](#) and only brief details are repeated here. The crop was ratooned (allowed to regrow) after harvesting the plant crop on 23 August 2000 and was irrigated and fertilized according to industry recommendations for achieving potential yields. On 22 October 2000, four recently calibrated tube solarimeters (1 m long) were placed on the soil surface in two places near the BREB installation, to span the 1.8 m dual crop row configuration exactly. Two more tube solarimeters were mounted above the canopy so that the fraction of intercepted radiation could be determined.

On 17 September 1998, an automatic weather station (AWS) was installed in an open grassed area about 1 km from the BREB system at Kalamia. All components were described by [Inman-Bamber and McGlinchey \(2003\)](#). AWS data were used to determine daily reference evapotranspiration (ET₀) from [Allen et al. \(1998\)](#).

Total above ground biomass was determined on seven occasions during the development of the crop at Kalamia. All plant material was removed from one 18 m² quadrat in each of four sampling sites on each occasion. Shoots and stalks were counted and then weighed altogether. A sub sample of stalks was also weighed and then partitioned into green leaf, sheath plus immature stem, mature stem and dead leaf components. A sub sample of each of these components was weighed and then dried to constant mass in a forced draught oven set at 80 °C. Stalk and crop heights were estimated based on a stalk diameter of 23 mm, and leaves extending an additional 2 m above the stalks.

2.3. Modelling

We used a customised version of the model from WaterSense (Armour et al., 2012) to simulate the effects of elevated CO₂ on the field experiment above. WaterSense was a web-based irrigation scheduling service (now disabled) based on a model that used the most appropriate components of APSIM and Canegro for the purpose of helping sugarcane farmers to manage irrigation (Haines et al., 2008). Canegro and APSIM-Sugar differ considerably in regard to the transpiration process. Potential transpiration (T_0) in APSIM is determined by the amount of radiation intercepted (R_i), RUE, TE and the vapour pressure deficit (VPD) (Eq. (1)). Actual transpiration (T_A) is limited either by T_0 or the rate of root water supply to the crop (W_R) (Eq. (2)). The ratio of T_A to T_0 (0–1) is the measure of water stress affecting biomass gain directly and leaf expansion proportionally (Keating et al., 1999).

$$T_0 = RUE \cdot R_i \cdot VPD / TE \quad (1)$$

$$T_A = \min(T_0, W_R) \quad (2)$$

Older versions of Canegro use the Penman-Monteith (PM) equation in a procedure which includes a daily estimate of the canopy height (z_c) and leaf area index (LAI) (Inman-Bamber et al., 1993), but newer versions use the FAO56 approach (Allen et al., 1998) based on crop factors (Singels et al., 2008). The crop factor, also called the crop coefficient (K_c), is the ratio of crop transpiration plus evaporation from the soil (evapotranspiration or ET_c), to a reference evapotranspiration of a well-watered, short grass (ET_0) and is often regarded as a simple function of crop development alone (Allen et al., 1998).

For this study we used WaterSense logic (Armour et al., 2012) but we replaced the crop coefficient approach with the one in which evapotranspiration estimates vary with z_c and LAI as reported by Inman-Bamber and McGlinchey (2003) and Inman-Bamber et al. (1993, 2005). In this procedure latent heat flux is derived from the PM equation (Eq. (3)) and functions for canopy resistance, aerodynamic resistance and wind speed (Eqs. (4)–(6)). This was done to allow estimates of leaf conductance obtained from the glasshouse study to be scaled up to an estimate of canopy conductance of a field crop.

Latent heat flux from sugarcane transpiration is:

$$\lambda T_{cane} = (\Delta(R_n - G) + \rho c_p VPD / r_a) / (\Delta + \gamma(1 + r_c / r_a)) \quad (3)$$

Canopy resistance is:

$$r_c = r_s / (0.5LAI) \quad (4)$$

Aerodynamic resistance is:

$$r_a = (\ln((z_2 - 0.7z_c) / 0.026z_c))^2 / (u_2 \kappa^2) \quad (5)$$

where:

T_{cane} = potential transpiration for sugarcane (mm d⁻¹)
 c_p = specific heat of air at constant pressure (J kg⁻¹ K⁻¹)
 Δ = slope of the vapour pressure curve (kPa °C⁻¹)
 G = soil heat flux density (MJ m⁻² day⁻¹)
 γ = psychrometric constant (kPa °C⁻¹)
 κ = von Karmans's constant = 0.41
 λ = latent heat of vaporization of water (J kg⁻¹ °C⁻¹)
 R_n = net radiation (MJ m⁻² d⁻¹)
 ρ = air pressure (kPa)
 r_a = aerodynamic resistance (s m⁻¹)
 r_c = canopy surface resistance (s m⁻¹)

r_s = leaf resistance (s m⁻¹)

z_c = sugarcane canopy height (m)

The PM equation uses wind speed measured at 2 m and determines aerodynamic resistance to vapour flux as a function of the difference between crop height and the height of the wind speed measurement (Eq. (5)). Sugarcane can grow to heights well above 2 m thus preventing resolving this equation at times. Wind speeds (u_2) were therefore adjusted to a height of 10 m using the wind profile equation (Inman-Bamber and McGlinchey, 2003; Monteith and Unsworth, 2007):

$$u_2 = u_1 \cdot \ln((z_2 - d_r) / z_{or}) / \ln((z_1 - d_r) / z_{or}) \quad (6)$$

where:

u_1 and u_2 = wind speed at 2 and 10 m (m s⁻¹)

z_1 and z_2 = heights 2 and 10 m above the ground

d_r = zero plane displacement of reference surface = 0.07 m

z_{or} = roughness length of reference surface = 0.013 m

Thus T_{cane} is responsive to changes in LAI, crop canopy height and leaf (mainly stomatal) resistance (1/conductance). Here we are concerned only with the effect of CO₂ on stomatal resistance (r_s) even though this may influence LAI and canopy height (z_c) development indirectly through water supply and demand. Inman-Bamber and McGlinchey (2003) standardized LAI at 3.5 and r_s at 100 s m⁻¹ to account for daily evapotranspiration as measured in large weighing lysimeters. For these simulations we used the latter value for r_s for CO₂ levels (~325 ppm) at the time of the lysimeter measurements in the late 1960's (Thompson, 1986). r_s was allowed to increase at 12 s m⁻¹ for every 100 ppm increase in CO₂ based on the glasshouse experiment results (presented below). LAI and z_c were allowed to vary with crop development but a maximum of 5 m was allowed for z_c because of the tendency for sugarcane crops to lean or lodge when individual plants are longer than 5 m.

Evaporation from the soil (E_S) was derived as in WaterSense (Armour et al., 2012); based on the depth of water in the top soil layer in excess of the depth remaining after air drying (term 1 in Eq. (7)); and the fraction of radiation reaching the soil surface (remaining terms) (Eq. (8)). The factor (F) for transpiration at elevated, relative to current, levels of CO₂ is (Tubiello et al., 2000):

$$F = (\Delta + \gamma(r_{c0} + r_a) / r_a) / (\Delta + \gamma(r_{cf} + r_a) / r_a) \quad (7)$$

where:

r_{c0} = r_c at current CO₂ levels (s m⁻¹)

r_{cf} = r_c at elevated CO₂ levels (s m⁻¹)

Evaporation from the soil is (Armour et al., 2012):

$$E_S = E_0 \left(\left(\min((\theta_C - \theta_{AD}) / ((\theta_S - \theta_{AD}), 1.0)) \right)^3 (0.05 + \exp(-0.38LAI) - c) - 0.1(1 - \exp(-0.38LAI)) + 0.1 \right) \quad (8)$$

where E_0 is reference evapotranspiration (Allen et al., 1998); θ_C , θ_{AD} and θ_S are water contents for the soil on the day of calculation (θ_C), for air-dried soil (θ_{AD}) and for saturated soil (θ_S) and c is the fraction of the soil surface covered by cane residue (trash); $c = 0$ for this simulation (burnt cane).

Sugarcane actual evapotranspiration (ET_c in mm d⁻¹) is the sum

of soil evaporation and potential transpiration or root water supply whichever is the least (Eq. (9)).

$$ET_c = E_s + \min(T_{cane}, W_R) \quad (9)$$

The CO₂ level used in our simulations was 375 ppm ($r_s = 106 \text{ m s}^{-1}$) which was the 'current' level at the time of the BREB experiment by Inman-Bamber and McGlinchey (2003). The elevated CO₂ concentration was chosen as 720 ppm ($r_s = 148 \text{ m s}^{-1}$) to correspond with several experiments including ours, where the high CO₂ treatment was set at this level.

LAI was determined as in the APSIM-Sugar model (Keating et al., 1999) with leaf characteristics for the variety Q172 as in Table 1. APSIM interpolates between inflection points defined as 'x' and 'y' parameters (Table 1). For example the maximum area is 50 cm² for leaf #1 and it increases linearly to 500 cm² for leaf #12 and remaining leaves.

Dry above ground biomass accumulation (ΔB) was also based on the APSIM-Sugar model and the Canegro term for maintenance respiration ($M = 0.004$) (Eq. (10)).

$$\Delta B = \max(((W_R/T_{cane})(R_l \cdot S_A \cdot RUE) - M \cdot B), 0.0) \quad (10)$$

3. Results

3.1. Glasshouse experiment

Compared to previous CO₂ work on sugarcane using open top chambers, the glasshouse experiment provided improvements by using a demand-driven watering system to maintain treatment soil water levels irrespective of CO₂ treatments, and temperature and humidity control systems to compensate for the substantial reductions in transpiration and evaporative cooling under elevated CO₂. Nonetheless, as the first experiment in a new facility, there were some initial teething problems in fully regulating and matching conditions between the two chambers. Radiation and temperature conditions were very similar between chambers (Fig. 2), but humidity control was insufficient to fully offset the differences in transpiration between chambers and RH was slightly lower in the high than the low CO₂ chamber (Fig. 2). Analysis of variance for biomass yield per pot indicated that single effects of CO₂ level, variety and harvest date were statistically significant ($p < 0.02$) but no interactions were significant. The yield of above ground biomass was significantly higher in the low than the high CO₂ chamber (Fig. 3). Plants were watered on demand using the same soil moisture criteria, with frequent checks and watering, in

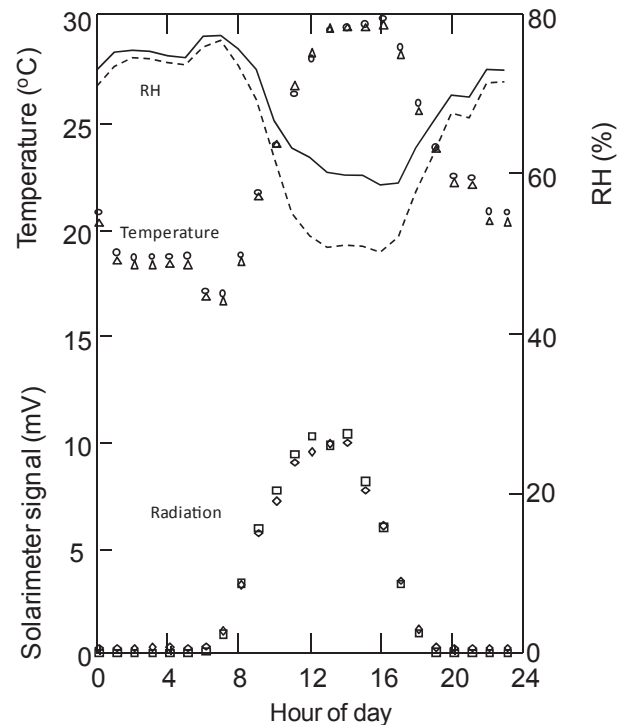


Fig. 2. Mean hourly RH (—, ---), temperature (○, △) and solarimeter voltage (radiation: □, ◇) for glasshouse chambers set to ambient (390 ppm; —, ○, □) or elevated CO₂ levels (720 ppm; ---, △, ◇).

both chambers so it is not likely that the lower RH in the high CO₂ chamber would have reduced yield through water stress. Mite damage was noted on leaves when gas exchange measurements were made shortly before the final harvest. This damage was confined to the high CO₂ chamber and may have contributed to the slightly lower yields at 8 and 10 months in this chamber. No mites were evident earlier in the experiment and the yields at 4 and 6 months would have benefited from exposure to elevated CO₂ had there been any direct effect of CO₂ on photosynthesis (see Fig. 3). The CO₂ effect on yield was not significant ($p = 0.108$) when the analysis of variance was confined to yields from these two earlier harvests, when no mites were present.

Variety, harvest date and CO₂ level all had significant effects ($p = 0.022$, $p < 0.001$ and $p < 0.001$ respectively) on whole-pot transpiration per unit leaf area over the 7-day period prior to

Table 1
Trait parameters for variety Q172 for determining leaf area index (LAI) and biomass gain as in the APSIM-Sugar model (Keating et al., 1999) and maintenance respiration as in Canegro (Singels and Bezuidenhout, 2002). APSIM terms are provided in parenthesis where applicable.

Trait (APSIM term)	Parameters				Units
Shoot population (plants)	10				per m ²
Thermal time for sprouting (shoot_lag)	100				Heat units (base 9 °C)
Planting depth (sowing_depth)	100				(mm)
Maximum green leaf number per stalk (green_leaf_no)	8				
Leaf number (xleaf_size)	1	12	40		
Leaf area (yleaf_size)	50	500	500		cm ²
Leaf number (xleaf_till)	1	4	10	16	
Leaf area multiplier for tillers	1.5	1.5	1.5	1	
Leaf number (x_node_no_leaf)	1	20	30	40	
Phyllochron (y_node_app_rate)	90	140	150	160	Heat units (base 9 °C)
Maintenance respiration fraction (M)	0.004				
Assimilation stress temperature (x_ave_temp)	5	15	35	50	°C
Assimilation temperature stress factor (S _A) (y_stress_photo)	0	1	1	0	

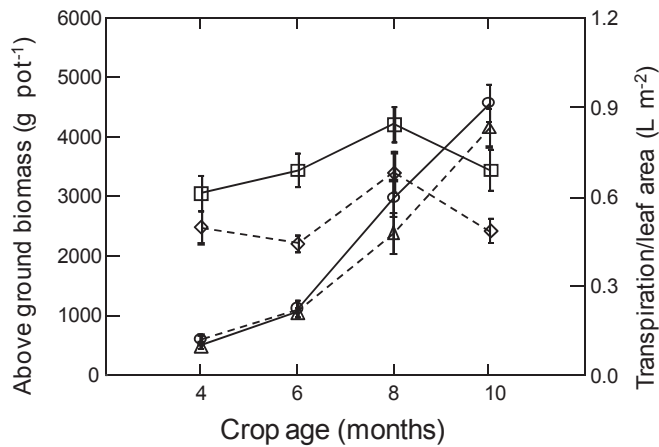


Fig. 3. Above ground biomass (○, △) and transpiration per unit leaf area (◇, □) of potted sugarcane plants growing in ambient (390 ppm; ○, □) or elevated CO₂ (720 ppm; △, ◇). Data are means for two varieties and four replicates and bars are 2x standard error.

sampling. However no interactions were significant. Elevated CO₂ reduced mean transpiration by 27.5% (Fig. 3.) and this, combined with inadequate humidifier-dehumidifier control, was probably the cause of the lower RH in the high than the low CO₂ chamber (Fig. 2.).

Cuvette (leaf chamber) measurements of gas exchange on leaf #1 indicated that photosynthesis was not affected with mite damage ratings of 1 (no damage) and 2 (slight damage within the range of natural blemishes, <5% of leaf area) (damage scores ranged from 1 to 5). Measurements on leaves where mite damage ratings exceeded natural blemishes (>2) were excluded from the analysis. Photosynthesis was increased ($p < 0.001$) by a temporary increase in CO₂ concentration in the cuvette regardless of whether plants had been growing at ambient or elevated CO₂ levels or not (Table 2). The cuvette × chamber interaction was not significant ($p = 0.085$) but there was a tendency for the response to an increase in cuvette CO₂ level to be greater for plants growing at ambient CO₂ levels than at elevated levels (Table 2). This was most likely due to the rather low internal CO₂ concentration (C_i) of leaves in ambient CO₂ (390 ppm) when cuvette CO₂ was 375 ppm (Table 2). For plants growing in elevated CO₂, C_i was also below the level (~100 ppm) thought to be saturating for C₄ species (Ghannoum et al., 2000) when the small cuvette CO₂ concentration was 375 ppm (Table 2).

The mean effect of an increase in cuvette CO₂ concentration on stomatal conductance was substantial (34% reduction, $p < 0.001$) and there was a tendency ($p = 0.054$) for the reduction to be greater

Table 3

Transpiration measurements for four days before and four days after swapping pots and CO₂ treatments between glasshouse Chambers, to separate CO₂ from Chamber effects.

Chamber No.	CO ₂ Treatment (ppm)	Transpiration (L/pot/day)
Immediately prior to swapping chambers and CO ₂ treatments		
1	720	1.09
2	390	1.83
Immediately after to swapping Chambers and CO ₂ Treatments		
1	390	1.68
2	720	1.10
Means for Chambers and CO ₂ Treatments		
Chamber 1		1.38
Chamber 2		1.46
	390 ppm CO ₂	1.75
	720 ppm CO ₂	1.09
ANOVA p values for main effects (no interactions were significant, $p > 0.05$)		
CO ₂		<< 0.001
Clone		<< 0.001
Chamber		0.45
Block		0.02

for plants growing at elevated, rather than at ambient, CO₂ (Table 2). However the more meaningful response is that between low CO₂ in both cuvette and chamber compared to high CO₂ in both types of chambers (i.e., when gas exchange measurements are made at the same CO₂ levels as the long term CO₂ treatments in which plants have been growing). This is more likely to be a measure of the long-term effect of CO₂ on conductance than any other comparison. In this case, it appears that long-term exposure to elevated CO₂ had reduced stomatal conductance by 28% which is very similar to the reduction in transpiration determined for all leaves over a 7-d period as discussed earlier.

We would expect that this reduction in transpiration was due largely to decreased stomatal conductance given that air-flow; radiation and temperature conditions as well as the 'canopy' structure in both large chambers were similar. Transpiration in the high CO₂ chamber may have been lower than observed had the RH been the same as in the low CO₂ chamber (Fig. 2). However a conservative approach would be to assume that conductance was reduced by 28% by long term exposure to elevated CO₂. This corresponds to a 39% increase in stomatal resistance to gaseous diffusion (r_s) (since r_s is the inverse of conductance) (Table 2) from prolonged exposure to elevated CO₂ (390 vs. 720 ppm). Thus r_s increased 11.8% per 100 ppm increase in CO₂.

Brief clarifying results from the later experiment, in which problems with environmental controls and mites were addressed, confirmed that CO₂ responses were predominantly restricted to reductions in water use, and that these changes in water use were

Table 2

Photosynthesis, leaf conductance and internal CO₂ concentration of sugarcane growing with ambient (390 ppm) or elevated (720 ppm) CO₂, supplied temporarily with ambient (375 ppm) or elevated CO₂ (720 ppm) in a small cuvette. Data for two varieties were pooled.

CO ₂ concentration (ppm)		Photosynthesis rate (μmol/m ² /s)	Stomatal conductance (mmol/m ² /s)	Internal CO ₂ concentration (ppm)
Cuvette (temporary CO ₂ treatment)	Chamber (long term CO ₂ treatment)			
375	390	25.4	161	66
375	720	28.4	196	81
375	Mean	26.9	179	74
720	390	33.8	120	178
720	720	31.4	116	189
720	Mean	32.6	118	184
ANOVA p values				
Cuvette		<0.001	<0.001	<0.001
Chamber		0.818	0.114	0.435
Chamber x Cuvette		0.085	0.054	0.888

Table 4

Final harvested aboveground biomass for the second experiment.

CO ₂ treatment (ppm)	Aboveground biomass (g dry mass/pot)
390	732
720	692
ANOVA p values for main effects (no interactions were significant, $p > 0.05$)	
CO ₂	0.52
Clone	<0.001
Block	0.52

the result of CO₂ treatments rather than other differences between chambers. Measurements of whole-pot transpiration immediately before and after swapping pots and CO₂ treatments between chambers (Table 3) showed significant differences between CO₂ treatments and clones (both $p < 0.01$), but not between chambers ($p = 0.45$). Final harvested aboveground biomass showed differences between clones ($p < 0.01$), but not between CO₂ treatments ($p = 0.52$, Table 4).

3.2. Modelling the field experiment at current and elevated CO₂ levels

The simulated fraction of solar radiation (400–7000 nm) reaching the soil surface was similar to what was observed using the tube solarimeters (Fig. 4). A close approximation of radiation levels at the soil surface was important for the correct partitioning of latent heat flux between evaporation from the soil (Eq. (8)) and transpiration from the canopy.

Simulated ET_c provided a good approximation of ET_c measured with the BREB system (Fig. 5). The process for deciding if ET_c measurements logged at 20 min intervals were deemed acceptable or not depending on wind direction and the resolution of the sensors (Inman-Bamber and McGlinchey, 2003). If all 40 ET_c readings between 06:00 and 19:00 on a given day were acceptable, then the acceptance rate for cumulative ET_c on that day was 100%. The correlation between simulated and measured daily ET_c was high ($R^2 = 0.65$, $n = 127$) when an acceptance rate of 80% was used for measured ET_c and was very high ($R^2 = 0.83$, $n = 41$) when the acceptance rate was 90%. Thus the more reliable the measurements, the closer they were to those estimated by the model. In this case one would rely more on the model than the measurements when their acceptance rates were low.

The simulation of biomass yield was close to the yield observed by means of the seven sample harvests conducted during the growth of the crop. Observed biomass yield was similar to simulated biomass yield for three of the harvest samples and was lower than simulated yield for one sample and higher for three of the

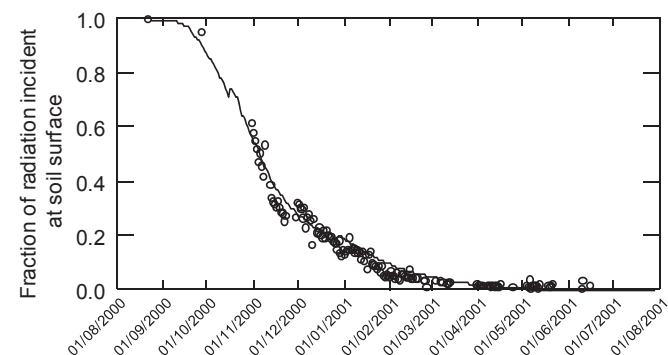


Fig. 4. Measured (○) and simulated (—) fraction of solar radiation reaching soil surface.

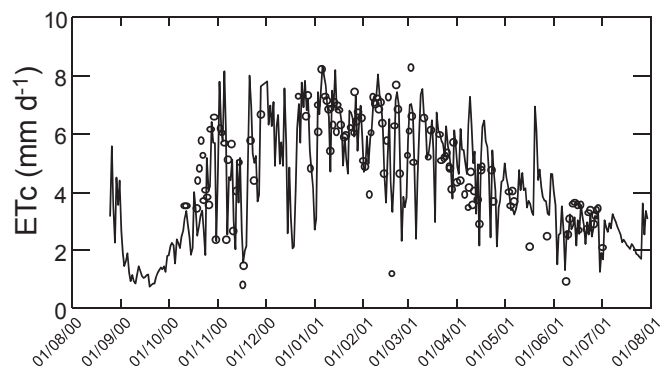


Fig. 5. Measured (○) and simulated (—) evapotranspiration (ET_c). Acceptance rate for measurements as defined by Inman-Bamber and McGlinchey (2003) was 80%.

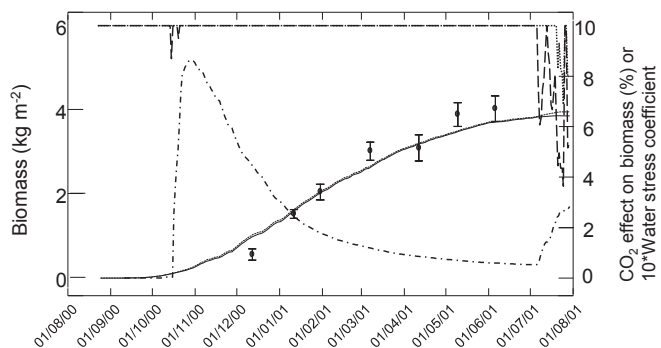


Fig. 6. Measured (●) and simulated daily crop dry biomass at current (—) and elevated (---) CO₂ levels, simulated response (biomass for elevated CO₂/biomass for current CO₂ * 100%) to increased CO₂ (---) and the daily water stress coefficient (root supply/transpirational demand) under current (—) and elevated CO₂ (....). Bars are 2 x standard error of the mean. (Field measurements are from Inman-Bamber and McGlinchey (2003)).

samples (Fig. 6). Although simulations of elevated CO₂ made little difference to biomass yield because this crop was well irrigated, biomass yield was 8% greater with elevated CO₂ than without it when the crop was young due to a short period of water stress in October 2000, and it was 3% greater at harvest (August 2001), also because of water stress caused by the drying off process which limited water availability to the crop. Reduced water use due to elevated CO₂ resulted in more water being available during the stress periods (Fig. 6).

Simulated LAI corresponded with measured LAI when this was

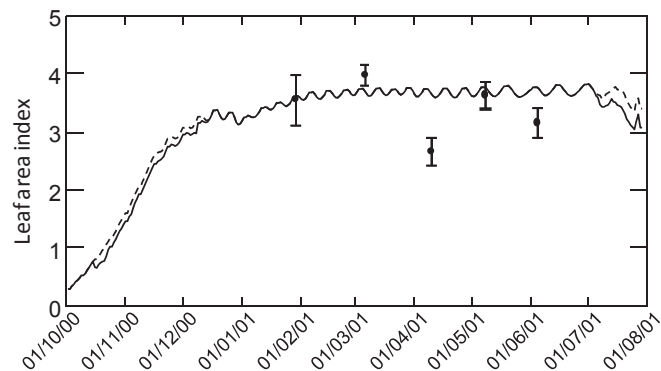


Fig. 7. Measured (●) and simulated leaf area index (LAI) at current CO₂ levels (—) and elevated (---) CO₂ levels. Bars are 2 x standard error of the mean. (Measurements are from Inman-Bamber and McGlinchey (2003)).

determined for the first time in January 2001 (Fig. 7). LAI observed in March and May also supported the assumptions about canopy development processes in the model. Measurements of LAI in April and June were lower than simulated LAI. The low LAI in April was unexpected but the reduction in LAI in June was possibly due to drying off prior to harvesting – a process not reflected in the simulation until July (Fig. 7). Canopy development would have benefitted from elevated CO₂ to only a small extent during the short-lived stress period in October 2000 (Fig. 7).

The simulation of stalk length was realistic (Fig. 8). Crop height (stalk height plus leaf length) was not measured as such but a maximum canopy height of 5 m was realistic given the 4 m height of the scaffolding built to service the BREB sensors and observations that leaves extended only about 1 m above that platform when the crop was at its tallest. Crop canopy height (z_c) and LAI need to be correctly modelled since these terms are used in the PM calculation of transpiration (Eqs. (3)–(5)).

The ratio (F) of transpiration at 720 ppm CO₂ relative to transpiration at 375 ppm CO₂ was as low as 0.7 when LAI was small and F increased to a range of 0.8–0.95 (Fig. 9) as LAI reached 3.5 and crop height 5 m (Figs. 7 and 8). F increased to as much as 1.5 during the drying-off period in July because high CO₂ and consequently reduced transpiration, increased the amount of water stored in the soil which could then be transpired during this period more readily than the crop subjected to low CO₂. When LAI and canopy height (z_c) are small, r_a is small and the effect of CO₂ on leaf resistance (r_s) has a large flow-on effect on transpiration (Eqs. (4), (5) and (7)). When LAI and z_c are large, r_a is relatively large so the impact of elevated CO₂ on transpiration is reduced when water is not limiting.

The ratio of ET_c to ET_0 is the crop coefficient (K_c) as defined by Allen et al. (1998). Measured and simulated K_c were similar and in agreement with maximum $K_c = 1.25$ as in Allen et al. (1998) and Inman-Bamber and McGlinchey (2003). However it is clear from the measurements and simulation that K_c for sugarcane is not constant but varies with water content at the soil surface, with crop height and with LAI and with climatic factors (wind speed). Simulated and measured K_c varied mostly between 1.0 and 1.5 indicating that irrigation scheduling based on simple models using constant K_c values could be flawed particularly if irrigation is applied daily in sandy soils. LAI for sugarcane in the BREB experiment was as high as 4.0 compared to an effective LAI = 1.44 for grass in the reference ET_0 calculation (Allen et al., 1998). Crop height exceeded 4 m for sugarcane in the BREB experiment compared to a grass height of 0.12 m in the ET_0 calculation (Allen et al., 1998). Stomatal resistance for grass (Allen et al., 1998) and

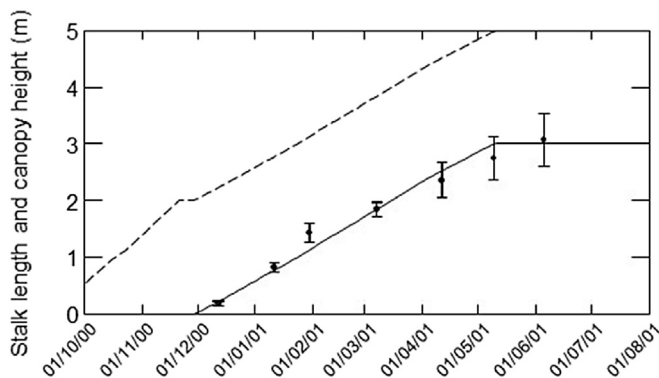


Fig. 8. Observed stalk length (●) derived from measured fresh stalk mass and an estimate of stalk diameter and simulated stalk length (—). Simulated crop canopy height (z_c ; - - -) accounts for the approximate 2 m that leaves extend above the top of the stalks.

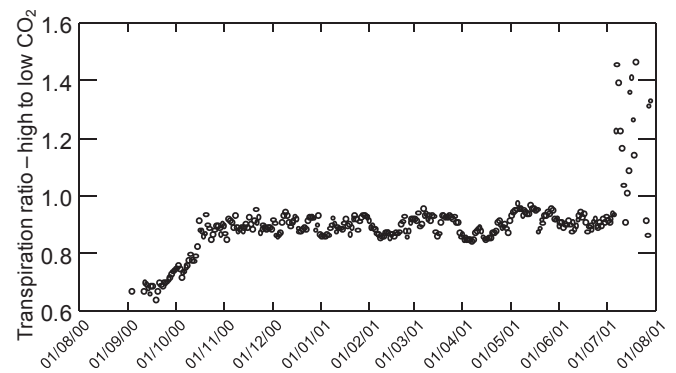


Fig. 9. Simulated ratio (F) of transpiration at 720 ppm CO₂ relative to transpiration at 375 ppm CO₂ for the duration of the field experiment, as the plants mature and the canopy closes.

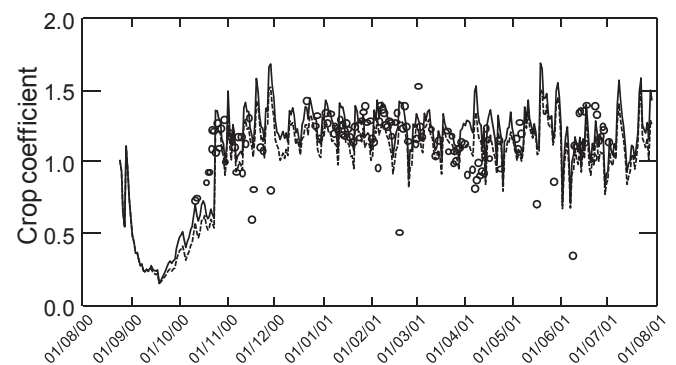


Fig. 10. Measured (○) and simulated daily crop coefficient (K_c) at current (—) and elevated CO₂ levels (---).

sugarcane was similar (100 and 106 s m⁻¹, respectively) at current CO₂ levels and was 148 s m⁻¹ for sugarcane at elevated CO₂ levels and this increase caused a slight reduction in K_c for sugarcane growing at twice normal CO₂ (Fig. 10).

4. Discussion

The glasshouse experiment presented here provides evidence that the direct stimulation of sugarcane growth by elevated CO₂ from purely leaf-level mechanisms, if any, is small. This suggests that reported increases in yield from previously published CO₂ experiments (Vu et al., 2006; de Souza et al., 2008) are likely to be largely due to indirect mechanisms related to improved water relations (the alleviation of water stress and prolonged soil water availability) even if water was thought to have been non-limiting during the experiments. Better water relations could have explained the results of Vu et al. (2006) where photosynthesis of small sugarcane leaf segments of young leaves was 10–20% greater in 720 ppm than ambient ppm CO₂ because elevated CO₂ reduced stomatal conductance by 51% and transpiration by 39%. Vu et al. (2006) suggested that the increase in leaf area (31%) and stalk yield (55%) with elevated CO₂, could have been partly through enhanced water use efficiency and therefore stress alleviation and prolonged water availability. de Souza et al. (2008) clarified their efforts to ensure adequate irrigation by maintaining soil water tension below 20 kPa and yet the 40% increase in biomass yield under elevated CO₂ was thought to be at least partly due water stress alleviation.

Interestingly, even without any direct effect of CO₂ on

photosynthesis, the modelling of the BREB experiment showed a biomass yield advantage as high as 8%, early in the accumulation of biomass by the crop (Fig. 6) which we thought at the time was irrigated adequately. However the final yield of the experiment would have increased only by about 3% at 720 ppm CO₂ according to the simulation. Marin et al. (2013) simulated a 10% increase in fresh cane yield with 750 versus 380 ppm CO₂ in their study using climatic conditions in Sao Paulo state, Brazil where irrigation is generally not practiced but rainfall is high. Their model was DSSAT/Canegro which uses a crop factor (K_c) approach based on the Penman-Monteith formula to determine grass reference evapotranspiration (ET_0) where canopy resistance for 'grass' was allowed to vary with CO₂ (Marin et al., 2013). However sugarcane evapotranspiration could not be influenced by the interaction between CO₂ concentration, crop height and LAI as we suggest it should be. Another problem with the crop coefficient (K_c) approach is that K_c varies considerably depending on soil moisture and various climatic variables (Fig. 10). Relative humidity, wind speed and crop height also need to be taken into account as we have done or as suggested by Allen et al. (1998) in the 'Dual crop coefficient' approach. A similar problem with K_c was identified in the CropSyst model by Marsal et al. (2014) using lysimeters rather than a BREB system. Furthermore, simulation of the BREB experiment with 375 and 720 ppm CO₂ indicated that transpiration could be reduced by about 30% when the crop is small but only by about 10% when the canopy is closed and the crop is tall (Fig. 9). Similar simulations were produced by Morison and Gifford (1984b) who plotted F (the ratio of transpiration under elevated vs ambient CO₂) against the ratio of r_a to r_s for different responses of r_s to the doubling of CO₂ concentrations. F was as low as 66% (when r_a/r_s was small, 0.04) and was as high as 0.90 (when r_a/r_s was 0.96 and stomatal conductance was reduced by 36%) for a doubling of CO₂ concentration. These authors point out, as we do, that the effect of stomatal closure on transpiration depends on the ratio of stomatal (r_s) to aerodynamic resistance (r_a). Only when stomatal resistance is large relative to aerodynamic resistance is the reduction in stomatal conductance with increased CO₂ concentration reflected as a reduction of similar magnitude in transpiration (Morison and Gifford, 1984b). In our case when simulating the BREB experiment, a reduction of 29% in stomatal conductance (a 40% increase in r_s) resulted in a 30% reduction in transpiration only when the crop was small (Fig. 9) and well-coupled with the atmosphere ($r_a \ll r_s$). For models of canopy transpiration that do not use a full Penman-Monteith (Eq. (3)) approach, it is important to take this decoupling and diminished response into account when scaling and parameterising canopy level CO₂ responses from leaf-level measurements. This is particularly important for crops like sugarcane where the canopy is closed, and r_a is large, for most of the crop's growth.

If changing levels of CO₂ affect crop growth only through alleviation of water stress then clearly the degree of water stress encountered by the crop under current CO₂ conditions will influence the degree to which the crop responds to CO₂. Inman-Bamber and Smith (2005) show how sensitive sugarcane is to water stress in terms of expansive growth. Inman-Bamber et al. (2008) showed how an increase in temperature for plants growing in a glasshouse can cause a reduction in leaf extension at midday presumably through water stress even when plants have water 'on demand' as was the case in our glasshouse experiment. Depending on the variety, hourly photosynthesis may not be reduced at all or by 50% at most by water stress which is sufficient to stop leaf extension (Inman-Bamber et al., 2008) so it was not surprising that our glasshouse grown plants did not respond to elevated CO₂ in terms of increased biomass. Growth of leaves of *Panicum coloratum* growing in controlled environment chambers was greater when elevated CO₂ (1000 ppm) decreased stomatal conductance and

transpiration under high VPD conditions, even though soil water content was maintained at 100% (Seneweera et al., 1998). These authors concluded that greater CO₂ concentrations allow C₄ grasses to maintain better internal water relations by reducing transpirational water losses and so allow expansion of these species into more arid climates. In a simulation study on possible sugarcane yields in Ghana, Black et al., 2012 found that a doubling of atmospheric CO₂ concentration offset a 20% increase in demand for irrigation associated with a 4 °C rise in temperature. The model used a simple approach for representing direct effects of CO₂ on plant water use whereby changes in stomatal conductance were assumed to be inversely proportional to changes in CO₂ level (e.g. a halving of conductance for a doubling of CO₂: stronger than the actual measured responses for sugarcane summarised in the paragraph below).

A comprehensive review on the effects of elevated CO₂ on C₄ species by Leakey (2009) sides with conclusions by Ghannoum et al. (2000) that C₄ photosynthesis could only be stimulated by elevated CO₂ either directly, when internal CO₂ concentration (C_i) is below about 100 ppm or indirectly, when reduced stomatal conductance stimulated photosynthesis via altered water relations or energy balance. Of the 220 C_i measurements (not published) on sugarcane growing at normal CO₂ levels, taken by Inman-Bamber et al. (2008) only seven C_i readings were less than 100 ppm; six of these were for water stressed plants. Mean C_i was significantly lower (120 ppm) for the dry treatments compared to the wet treatment (198 ppm, $p = 0.014$). In another experiment in which potted sugarcane had water on demand, whole plant photosynthesis (per unit leaf area) declined about 66% from 6 to 10 months of age (Inman-Bamber et al., 2011). Bull, 1969 reported a similar decline in photosynthesis of leaf segments for plants between 10 and 48 weeks of age. Basnayake et al. (2015) measured stomatal conductance on various irrigated and rainfed crops during the first six months of development. For irrigated crops the mean final conductance was 202 mmol/m²/s compared to 259 mmol/m²/s for the earlier conductance's. In our experiment (Table 2), mean conductance in normal CO₂ was 179 mmol/m²/s in the 9-month-old plants, which is probably to be expected for well-watered plants of that age. The low C_i values in Table 2 are probably due to the rather low stomatal conductance related to crop age rather than to water stress. Thus ageing crops could respond to elevated CO₂ directly through increased C_i but this was not evident in the biomass yields of our experiment.

The most consistent effect of elevated CO₂ reported in the sugarcane literature is that on stomatal conductance; a 37% and 34% reduction in twice normal CO₂ (de Souza et al., 2008; Vu et al., 2006) and in our case a 28% reduction for well-watered plants when CO₂ concentration was elevated from 390 to 720 ppm (Table 2). This agrees with research from other C₄ species such as maize (Leakey et al., 2006) where, in the absence of water stress, growth under elevated CO₂ (550 ppm compared to 367 ppm) did not stimulate photosynthesis, biomass accumulation or yield. Nor was there any CO₂ effect on the activity of key photosynthetic enzymes, or metabolic markers of carbon and nitrogen status (Leakey et al., 2006).

It behoves modellers to be careful about assumptions used when representing CO₂ effects in their models (Tubiello et al., 2007) and experimental evidence has been lacking up to now for simulating the response of sugarcane yields to future rises in CO₂ concentrations. In this paper we presented initial experimental evidence that indicates modelling future climate scenarios for sugarcane should be based on representing CO₂ responses predominantly through indirect water-related mechanisms.

We also provided a modelling framework that demonstrates the value of using carefully targeted, controlled studies to test and

isolate proposed response mechanisms and subsequently reconstruct (scale up) their combined action under a field crop situation, through modelling. Algorithms developed in the simulation study are well suited for investigating possible responses of sugarcane to climate change scenarios in which CO₂, radiation, temperature and rainfall may all change, and have been applied in this way by Everingham et al. (2015).

Acknowledgements

This work was jointly funded by the Sugar Research and Development Corporation (Project No. CPI018), Sugar Research Australia (Project No. CPI018) and the authors' home institutions. Technical assistance with this project was provided by Michael Hewitt, Paul McLennan, Peter Bakker, David Boadle and Justin Perry.

Abbreviations

A	CO ₂ assimilation from photosynthesis
BREB	Bowen ratio energy balance
C _i	internal CO ₂ concentration of leaves
ET _c	evapotranspiration
K _c	crop coefficient; crop factor
LAI	leaf area index
PM	Penman-Monteith
RH	relative humidity
RUE	radiation use efficiency
r _a	aerodynamic resistance
r _s	in stomatal resistance to gaseous diffusion
TE	intrinsic transpiration efficiency
VPD	vapour pressure deficit

References

- Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration – Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. FAO – Food and Agriculture Organization of the United Nations, Rome.
- Armour, J., Nelson, P., Daniells, J., Rasiah, V., Inman-Bamber, N., 2012. Nitrogen leaching from the root zone of sugarcane and bananas in the humid tropics of Australia. *Agric. Ecosyst. Environ.* 180, 68–78.
- Asseng, S., Jamieson, P.D., Kimball, B., Pinter, P., Sayre, K., Bowden, J.W., Howden, S.M., 2004. Simulated wheat growth affected by rising temperature, increased water deficit and elevated atmospheric CO₂. *Field Crop. Res.* 85 (2–3), 85–102.
- Basnayake, J., Jackson, P.A., Inman-Bamber, N.G., Lakshmanan, P., 2015. Sugarcane for water-limited environments. Variation in stomatal conductance and its genetic correlation with crop productivity. *J. Exp. Bot.* 66 (13), 3945–3958. <http://dx.doi.org/10.1093/jxb/erv194>.
- Biggs, J.S., Thorburn, P.J., Crimp, S., Masters, B., Attard, S.J., 2013. Interactions between climate change and sugarcane management systems for improving water quality leaving farms in the Mackay Whitsunday region, Australia. *Agric. Ecosyst. Environ.* 180, 79–89.
- Black, E., Vidale, P.L., Verhoef, A., Cuadra, S.V., Osborne, T., Van den Hoof, C., 2012. Cultivating C₄ crops in a changing climate: sugarcane in Ghana. *Environ. Res. Lett.* 7 (4), 044027.
- Bull, T.A., 1969. Photosynthetic efficiencies and photorespiration in Calvin cycle and C₄-dicarboxylic acid plants. *Crop Sci.* 9 (6), 726–729.
- de Souza, A.P., Gaspar, M., Da Silva, E.A., Ulian, E.C., Waclawowsky, A.J., Nishiyama Jr., M.Y., Dos Santos, R.V., Teixeira, M.M., Souza, G.M., Buckeridge, M.S., 2008. Elevated CO₂ increases photosynthesis, biomass and productivity, and modifies gene expression in sugarcane. *Plant Cell Environ.* 31 (8), 1116–1127.
- Everingham, Y., Inman-Bamber, G., Sexton, J., Stokes, C., 2015. A dual ensemble agroclimate modelling procedure to assess climate change impacts on sugarcane production in Australia. *Agric. Sci.* 6, 870–888.
- Ghannoum, O., Caemmerer, S.V., Ziska, L.H., Conroy, J.P., 2000. The growth response of C₄ plants to rising atmospheric CO₂ partial pressure: a reassessment. *Plant Cell Environ.* 23 (9), 931–942.
- Ghannoum, O., Conroy, J.P., Driscoll, S.P., Paul, M.J., Foyer, C.H., Lawlor, D.W., 2003. Nonstomatal limitations are responsible for drought-induced photosynthetic inhibition in four C₄ grasses. *New Phytol.* 159 (3), 599–608.
- Haines, M., Inman-Bamber, N., Attard, S., Linedale, A., 2008. Enhancing Irrigation Management Planning with EnviroScan and WaterSense. Irrigation Australia Limited Conference, Melbourne. http://irrigation.org.au/documents/publications-resources/conference_papers_2008/220508_Stream2_MGHaines.pdf [accessed 04/08/2013].
- Holzworth, D.P., Huth, N.I., deVoi, P.G., Zurcher, E.J., Herrmann, N.I., McLean, G., Chenu, K., van Oosterom, E.J., Snow, V., Murphy, C., Moore, A.D., Brown, H., Whish, J.P.M., Verrall, S., Fainges, J., Bell, L.W., Peake, A.S., Poulton, P.L., Hochman, Z., Thorburn, P.J., Gaydon, D.S., Dalglish, N.P., Rodriguez, D., Cox, H., Chapman, S., Doherty, A., Teixeira, E., Sharp, J., Cichota, R., Vogeler, I., Li, F.Y., Wang, E., Hammer, G.L., Robertson, M.J., Dimes, J.P., Whitbread, A.M., Hunt, J., van Rees, H., McClelland, T., Carberry, P.S., Hargreaves, J.N.G., MacLeod, N., McDonald, C., Harsdorf, J., Wedgwood, S., Keating, B.A., 2014. APSIM – evolution towards a new generation of agricultural systems simulation. *Environ. Model. Softw.* 62, 327–350.
- Inman-Bamber, N., Culverwell, T., McGlinchey, M., 1993. Predicting yield responses to irrigation of sugar cane from a growth model and field records. In: Proceedings of the South African Sugar Technologists' Association, pp. 66–72.
- Inman-Bamber, N.G., Bonnett, G.D., Spillman, M.F., Hewitt, M.L., Jackson, J., 2008. Increasing sucrose accumulation in sugarcane by manipulating leaf extension and photosynthesis with irrigation. *Aust. J. Agric. Res.* 59 (1), 13–26.
- Inman-Bamber, N.G., McGlinchey, M.G., 2003. Crop coefficients and water-use estimates for sugarcane based on long-term Bowen ratio energy balance measurements. *Field Crop. Res.* 83 (2), 125–138.
- Inman-Bamber, N.G., Smith, D.M., 2005. Water relations in sugarcane and response to water deficits. *Field Crop Res.* 92 (2–3), 185–202.
- Inman-Bamber, N.G., Jackson, P.A., Hewitt, M., 2011. Sucrose accumulation in sugarcane stalks does not limit photosynthesis and biomass production. *Crop Pasture Sci.* 62, 848–858.
- Keating, B.A., Robertson, M.J., Muchow, R.C., Huth, N.I., 1999. Modelling sugarcane production systems I. Development and performance of the sugarcane module. *Field Crop Res.* 61 (3), 253–271.
- Kiker, G., Inman-Bamber, N.G., Hoogenboom, G., McGlinchey, M., 2002. Further progress in the validation of the CANEGRO-DSSAT model. In: Proceedings of International CANEGRO Workshop: Mount Edgecombe, South Africa.
- Knox, J.W., Rodríguez Díaz, J.A., Nixon, D.J., Mkhwanazi, M., 2010. A preliminary assessment of climate change impacts on sugarcane in Swaziland. *Agric. Syst.* 103 (2), 63–72.
- Leakey, A.D.B., 2009. Rising atmospheric carbon dioxide concentration and the future of C₄ crops for food and fuel. *Proc. R. Soc. Lond. B* 276, 2333–2343.
- Leakey, A.D.B., Uribealarea, M., Ainsworth, E.A., Naidu, S.L., Rogers, A., Ort, D.R., Long, S.P., 2006. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO₂ concentration in the absence of drought. *Plant Physiol.* 140 (2), 779–790.
- Marin, F.R., Jones, J.W., Singels, A., Royce, F., Assad, E.D., Pellegrino, G.Q., Justino, F., 2013. Climate change impacts on sugarcane attainable yield in southern Brazil. *Clim. Change* 117 (1–2), 227–239.
- Marin, F.R., Thorburn, P.J., Nassif, D.S.P., Costa, L.G., 2015. Sugarcane model inter-comparison: structural differences and uncertainties under current and potential future climates. *Environ. Model. Softw.* 72, 372–386.
- Marsal, J., Johnson, S., Casadesus, J., Lopez, G., Girona, J., Stöckle, C., 2014. Fraction of canopy intercepted radiation relates differently with crop coefficient depending on the season and the fruit tree species. *Agric. For. Meteorol.* 184, 1–11.
- Matsuoka, M., Furbank, R.T., Fukayama, H., Miyao, M., 2001. Molecular engineering of C₄ photosynthesis. *Ann. Rev. Plant Biol.* 52 (1), 297–314.
- Monteith, J., Unsworth, M., 2007. Principles of Environmental Physics. Academic Press, Burlington, MA.
- Morison, J.I.L., Gifford, R.M., 1984a. Ethylene contamination of CO₂ cylinders: effects on plant growth in CO₂ enrichment studies. *Plant Physiol.* 75 (1), 275–277.
- Morison, J., Gifford, R., 1984b. Plant growth and water use with limited water supply in high CO₂ concentrations. I. Leaf Area, water use and Transpiration. *Funct. Plant Biol.* 11 (5), 361–374.
- Park, S., Creighton, C., Howden, M., 2007. Climate Change and the Australian Sugar Industry, Impacts, Adaptation and R&D Opportunities, Brisbane.
- Seneweera, S.P., Ghannoum, O., Conroy, J., 1998. High vapour pressure deficit and low soil water availability enhance shoot growth responses of a C₄ grass (*Panicum coloratum* cv. *Bambatsi*) to CO₂ enrichment. *Funct. Plant Biol.* 25 (3), 287–292.
- Sinclair, T.R., 2012. Is transpiration efficiency a viable plant trait in breeding for crop improvement? *Funct. Plant Biol.* 39 (5), 359–365.
- Singels, A., Bezuidenhout, C.N., 2002. A new method of simulating dry matter partitioning in the CANEGRO sugarcane model. *Field Crops Res.* 78 (2), 151–164.
- Singels, A., Jones, M., van den Berg, M., 2008. DSSAT V4. 5-CANEGRO Sugarcane Plant Module: Scientific Documentation. South African Sugarcane Research Inst, Mount Edgecombe, South Africa.
- Singels, A., Jones, M., Marin, F., Ruane, A.C., Thorburn, P., 2014. Predicting climate change impacts on sugarcane production at sites in Australia, Brazil and South Africa using the Canegro model. *Sugar Tech.* 16 (4), 347–355.
- Thompson, G.D., 1986. Agrometeorological and Crop Measurements in a Field of Irrigated Sugarcane. Mount Edgecombe Research Report No. 5. SASA Experiment Station, Mount Edgecombe, South Africa, ISBN 0-620-10103-2, 244.
- Tubiello, F.N., Amthor, J.S., Boote, K.J., Donatelli, M., Easterling, W., Fischer, G., Gifford, R.M., Howden, M., Reilly, J., Rosenzweig, C., 2007. Crop response to elevated CO₂ and world food supply: a comment on “Food for Thought...” by Long et al., science 312:1918–1921, 2006. *Euro. J. Agron.* 26 (3), 215–223.
- Tubiello, F.N., Donatelli, M., Rosenzweig, C., Stockle, C.O., 2000. Effects of climate

- change and elevated CO₂ on cropping systems: model predictions at two Italian locations. *Euro. J. Agron.* 13 (2–3), 179–189.
- Vu, J.C.V., Allen Jr., L.H., Gesch, R.W., 2006. Up-regulation of photosynthesis and sucrose metabolism enzymes in young expanding leaves of sugarcane under elevated growth CO₂. *Plant Sci.* 171 (1), 123–131.
- Vu, J.C.V., Allen Jr., L.H., 2009. Growth at elevated CO₂ delays the adverse effects of drought stress on leaf photosynthesis of the C₄ sugarcane. *J. Plant Physiol.* 166 (2), 107–116.
- Webster, A.J., Thorburn, P.J., Roebeling, P.C., Horan, H.L., Biggs, J.S., 2009. The expected impact of climate change on nitrogen losses from wet tropical sugarcane production in the Great Barrier Reef region. *Mar. Freshw. Res.* 60 (11), 1159–1164.