SRDC Research Project Final Report

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Pathways to exploiting enhanced photosynthetic efficiency for higher sucrose and biomass yield.

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Project Reference №:   CSE023

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Statement of Confidentiality:

No part of this report is considered confidential.

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EXECUTIVE SUMMARY

Issue

Australia has one of the highest commercial cane sugar (CCS) levels in the world but unfortunately CCS appears to have plateaud at about 14% of fresh cane weight over the past 20 years. Up to now in breeding programs, increased fibre has been considered to have negative economic impacts because of adverse effects on sugar extraction and milling rate. It is possible that high fibre genotypes can produce higher biomass yields than high sucrose types because high sucrose content in the stalk may feedback negatively on photosynthesis either through end-product suppression or through sugar signalling compounds. This is now an assumption which is gaining acceptance through recent publications. Prior to this project this assumption had not been tested using high fibre and high sucrose clones. Feedback inhibition is also suspected to be the cause of the 'reduced growth phenomenon', a term applied to lower than expected biomass accumulation after a certain stage in crop development. This project aimed to establish the role of cane stalk sucrose in feedback inhibition of photosynthesis in order to reveal existing limitations to increasing sucrose content and biomass yield.

Methodology

Data from two glasshouse experiments, one field experiment and one pot experiment were used to help address the objectives of this research. As many as 20 clones representing a wide range in sucrose and fibre contents were used in these experiments. Other outputs were achieved through modelling, literature research and preparation of journal articles and book chapters.

Outputs

Our data supported previous conclusions about localised feedback on photosynthesis by sugars accumulating in the leaf resulting in reduced photosynthesis of small segments of individual young leaves. Photosynthesis declined with crop age and sucrose content increased but there was no evidence to suggest that photosynthesis declined *because* sucrose content increased. It is likely that biomass yields will increase if biomass is the target trait for selection rather than sucrose yield but increased biomass yield may not necessarily result in significantly reduced sucrose content and increased fibre content.

After finding that feedback inhibition was unimportant, other causes of reduced photosynthesis and reduced biomass accumulation with crop age, were considered. It is possible that respiration of labile sugars in the stalk could explain the reduced growth phenomenon which was most noticeable in the Ord River Irrigation Area. Published equations (from SRDC and other research) on sucrose accumulation and its respiration, accounted for growth slowdown in the Ord indicating that respiration may well be the cause of reduced growth. These equations were then used in a model to estimate biomass yields attainable in a production system that made use of rainfall only and where stools could survive for several months without rain. Under these conditions, mean simulated biomass yields were 15 to 20 t/ha in regions such as Mareeba, Charters Towers and Bowen but yields were close to zero in some years.

Also because feedback inhibition was found to be unimportant we considered other options for increasing CCS. These options included the re-evaluation of ripeners with a better understanding of ripener, water, temperature and genotype interactions possibly using controlled conditions. Also, the limited rate of genetic gain in mill CCS could be due to current biases in predicting economic value of varieties in breeding programs, because sound whole stalks are used as a measure of variety CCS thus ignoring important genetic variation in suckering and in other traits affecting extraneous matter. In the long term we need to get beyond the measured CCS limit (21%) by exploiting genes that are not yet in the commercial germplasm pool. Molecular intervention may be required to break internal physiological limits.

Impacts

The important finding on feedback will call into question research programs designed to find and then down-regulate signals and feedback pathways from stalk to leaf. Importantly the work also shows that increased biomass yield from sugarcane may not come at the expense of high sucrose content to any large extent. If respiration of sugars is confirmed to be an important limitation to yield increases then selection or genetic modification for reduced 'futile' cycling of sugars could be attempted.

Breeders indicated that this finding is important to them and somewhat encouraging in that selection for biomass yield will not necessarily change their selection procedures unduly. It is possible that selection for biomass will lead to higher fibre and lower CCS for other reasons.

Respiration of sucrose could be responsible for the reduced growth phenomenon that is now accepted to occur in sugarcane and is believed to be possibly due to feedback inhibition of photosynthesis. The research conducted in this project should help to correct this view and thus help to direct research into the real causes of reduced growth.

The project findings have contributed to a number of international papers, industry publications and book chapters.

BACKGROUND

Selection pressure for high sucrose content in sugarcane breeding programs has been intense world wide. This is particularly the case in Australia where the pricing formula provides additional incentives for growers to deliver crops with high sucrose content to the mill. Australia has one of the highest commercially recoverable sugar contents (measured as commercial cane sugar content or CCS) in the world but unfortunately CCS appears to have plateaud at about 14% of fresh cane weight over the past 20 years (F.O. Licht 2009). Until now raw sugar has been the predominant commodity produced from sugarcane with the exception of Brazil where about 50% of the total fermentable sugars (mainly sucrose) is used to produce ethanol for transport fuel (Moreira and Goldemberg 1999) representing approximately 24.5 billion litres produced in 2009 (RFA 2010). Despite the interest in ethanol, Brazil has also achieved a percentage of sugar recovery from cane delivered to the mill that now exceeds that of Australia (F.O. Licht 2009).

The residue following juice extraction is called 'bagasse', consisting mostly of fibre (48%) and water. Fibre in the form of bagasse has traditionally been regarded as a by-product of either sugar or ethanol production and is used as a source of energy in sugarcane mills, inefficiently by design normally, in order to avoid costs of its disposal. There is now an increasing interest in fibre as a cheap source of energy and one of the first responses to the need for renewable energy is to improve the efficiency of steam and electricity generation from sugarcane fibre (bagasse) in sugar mills such that excess power is delivered to the grid (Cheesman 2004).

Up to now in breeding programs, increased fibre has been considered to have negative economic impacts because of adverse effects on sugar extraction and milling rate, and fibre has been weighted negatively in selection indices (Wei *et al.* 2008). If the fibre component of sugarcane becomes more valuable in future, then this will change selection indices used in breeding programs, and focus greater

attention on higher total biomass production than in the past. In particular, material more closely related to the high fibre, low sucrose species *S. spontaneum* may become more commercially attractive. Clones of *S. spontaneum* have been reported with fibre content as high as 56% on a fresh weight basis. Interspecific hybrids with up to 33 % fibre content are being tested as 'fuel canes' in the West Indies since they reportedly produce up to 5 times more fibre yield than commercial sugarcane varieties (Rao and Kennedy 2004). Wang *et al.* (2008) evaluated progeny from 43 bi-parental crosses between sugarcane and *S. spontaneum* clones, against several commercial 'sucrose' cultivars and reported a doubling of stalk biomass in clones with dry matter content as high as 41% and fibre up to 29%, although only small plots were used and results need to be interpreted cautiously. These results align with those from the West Indies where 'energy cane' produced 51 compared to 19 t fibre/ha from 'sugarcane' (Leal 2007). Botha (2009) compared three strategies for improving the value of sugarcane for both food and fuel markets based simply on the heat of combustion of sucrose and fibre. More energy would be derived by improving fibre than sucrose content even without the benefits of increased photosynthesis and new technologies for ligno-cellulosic fermentation.

The physiological basis for claims of high biomass from energy canes with high fibre content has not been well documented. Some studies were reported on an interspecfic hybrid of *Saccharum spp* 'L79-1002', selected for its high biomass. This clone produced 170 t/ha fresh cane mass compared to only 50 t/ha for a conventional sucrose type variety (Breaux *et al.* 1974). Radiation use efficiency (RUE) was reported to be 1.24 g DM per MJ of total solar radiation and 1.3 g/MJ of radiation intercepted by leaves. The high biomass of L79-1002 compared to other C4 grasses (maize and sorghum) was attributed to the long duration of its growth rather than to high RUE (Viator 2010). Maximum RUE of conventional sugarcane varieties in Australia was 1.59 to 1.72 g/MJ and while average RUE was lower at about 1.45 g/MJ (Robertson *et al.* 1996) it was still greater than that reported for the energy variety, L79-1002.

It is possible that high fibre genotypes can produce higher biomass yields than high sucrose types because high sucrose may feedback negatively on photosynthesis either through end-product suppression or through sugar signaling compounds such as trehalose-6-phosphate (McCormick *et al.* 2009). Sucrose feedback inhibition was thought to be involved in higher rates of photosynthesis when sugarcane plants were modified to produce isomaltulose as well as sucrose (Wu and Birch 2007). Irvine (1975) measured higher rates of photosynthesis in *S. spontaneum* with low sucrose contents than commercial hybrids (*Saccharum spp.*) with high sucrose contents, possibly because of feedback inhibition. Direct genetic manipulation of photosynthesis by targeting key enzymes, could be frustrated

by the overproduction of a sugar signal that repressed expression of photosynthetic genes (Paul *et al.* 2001).

OBJECTIVES

Sugarcane production systems have one of the highest net energy outputs of all cultivated plant systems. This project aimed to establish the role of sucrose in feedback inhibition of photosynthesis in order to:

1.   Obtain a better understanding of the basic physiology of sugarcane and existing limitations to higher sucrose content and yield, which may lead to innovative approaches to crop improvement (conventional breeding or genetic engineering) in the future.

2.   Assess options for increasing the photosynthetic efficiency of sugarcane and enhancing its natural physiological advantages as a feedstock for the growing biofuel market

3.   Assess options for increasing sucrose accumulation rate by managing planting and harvesting to minimise any proven feedback inhibition or excessive respiration.

METHODOLOGY

Data from four experiments were used to help address the objectives of this research. Experiment 1 was part of an earlier project (CSE014) and some results from this experiment have been published along with a full description of the methodology (Inman-Bamber *et al.* 2009). Additional and reworked data from this experiment was used to assess feedback effects of sucrose content on photosynthesis. Experiment 2 was conducted in the field as part of this project (CSE023) and was followed by a glasshouse experiment (experiment 3) to assess physiological and morphological differences between clones chosen either for high fibre or high sucrose content. Experiment 4 was done with these same clones to compare morning and afternoon leaf photosynthesis in young plants to determine if localised feedback occurs before sucrose accumulates in stalks to any significant extent.

Choice of clones

For experiment 1 two high sucrose and two low sucrose clones were selected from a total of five commercial cultivars and 29 unselected clones from 3 biparental crosses (IJ76-514 X Q165, KQ99-1401X Mida, and MQ77-340 X Q117) as described by Inman-Bamber *et al.* (2009). The two low sucrose clones (C1 and C2) had fibre contents in the upper 15 percentile while the two high sucrose clones had

fibre contents lower than the median (Fig. 1). Sucrose content for the two low sucrose clones was in the lower 20 percentile and was in the upper 30 percentile for the two high sucrose clones (Fig. 1).
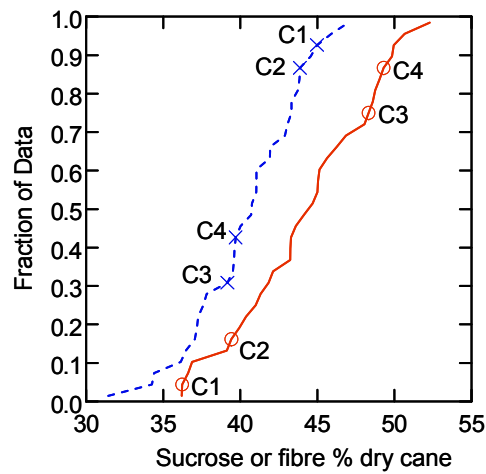
Fig. 1. Cumulative distributions for sucrose (solid line) and fibre (broken line) content of dry stalks for 32 clones, highlighting data for two low sucrose clones (C1 and C2) and two high sucrose clones (C3 and C4) in experiment 1.

Experiments 2 and 3 were based on a subset of 20 clones selected either for high fibre or high sucrose content of fresh mass from a set of 80 clones (Fig. 2) being assessed for variation in drought resistance in the Australian sugarcane improvement program. These 80 clones included commercial sugarcane varieties (*Saccharum* spp hybrids) as well as crosses between commercial sugarcane varieties and *S. spontaneum* and *Erianthus* spp (Wang *et al.* 2008; Jackson 2007).
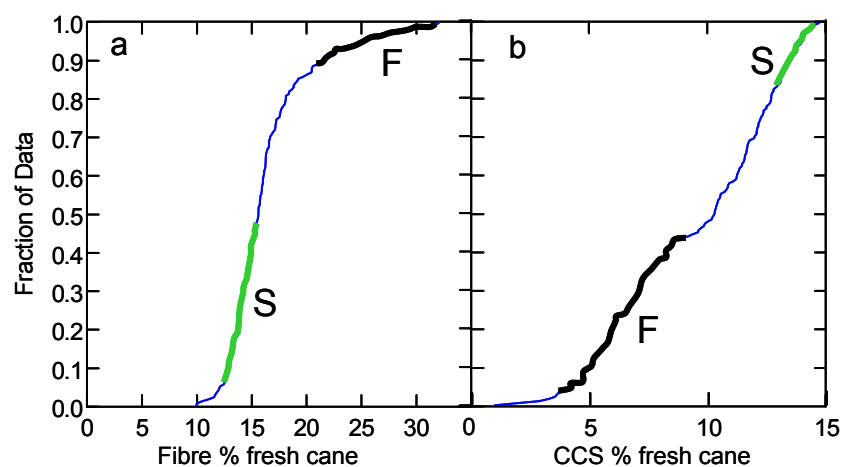
Fig. 2. Cumulative distributions functions for fibre % cane (a) and CCS % cane (b) for 80 clones and for the subset of clones (thickened lines) chosen for high sucrose content (S) or high fibre content (F).

The 10 clones selected for high fibre content ranked in the top 12% of the 80 clones. The fibre content of clones selected for their sucrose content was distinctly lower – 13 to 16% but the CCS of these clones was in the top 20% at 13 to 14% while the CCS of the fibre clones was only 3 to 8%. Seven of the high sucrose clones were commercial varieties but none of the fibre clones had been subjected to any selection pressure (Table 1). Only 18 of the 20 clones used in experiment 2 could be accommodated in the glasshouse for experiment 3 because of limited space so the lowest ranking clone in each of the fibre (F10) or sucrose (S10) groups was excluded.

Table 1. Codes in rank order (fibre or sucrose content) for clones and varieties of sugarcane used in experiment 3. The superscript 'A' signifies a commercial variety.

Fibre clones		Sucrose clones	
Code	Clone	Code	Clone
F01	CT04-450	S01	QS95-6004
F02	QB01-5	S02	CT05-735
F03	CT05-199	S03	Q117[A]
F04	CT04-28	S04	QN83-1072
F05	CT04-577	S05	Q207[A]
F06	CT04-845	S06	Q229[A]
F07	CT04-495	S07	KQ228[A]
F08	QB01-3	S08	Q209[A]
F09	CT04-559	S09	TELLUS[A]
F10	CT05-605	S10	Q171[A]

Cultural practices

The four clones of experiment 1 were planted in germination trays containing a sand/peat mixture on 11–13 Jul 2006 and then transplanted into 27-L pots filled with 25 kg of a commercial potting mix after about 3 leaves had emerged (Inman-Bamber *et al.* 2009). The pots were placed in the open and were irrigated 3-times a day. Fertiliser (Osmocote; Scotts Australia Pty Ltd, NSW, Australia; 14.0 : 6.1 : 11.6, N: P : K) was applied in ample amounts (35 g) to each pot at transplanting and at monthly intervals thereafter. On 20 Dec 06, 12 pots of each clone were transferred to each of two chambers in the glasshouse known as the 'CSIRO Tall Plant Facility' (TPF) described by Inman-Bamber *et al.* (2009). Irrigation was applied automatically to pots in one chamber so as to minimize water stress as detected by measuring plant extension on a continuous basis. Pots in the other chamber were subjected to moderate water stress by withholding water to achieve an average extension rate about half that of the

well watered plants. The irrigation treatments had to be applied to chambers separately because the chambers were used as large photosynthesis cuvettes to allow a comparison of photosynthesis rates between well watered and partially stressed plants (Inman-Bamber *et al.* 2009).

The 20 clones in experiment 2 were each grown in field plots 9 m long and 4 rows wide, 1.8 m apart. These plots were planted on 30 Jun 2007 as part of another project (BSS305) on variation in drought resistance in the Australian gene pool. The plots were machine harvested on 16 Jun 2008 to start the first ratoon crop. Irrigation was applied by furrow using the WaterSense scheduling software (Haines and Attard 2010) thus ensuring minimum water stress. Fertilizer was applied to deliver 130 kg N/ha and 13 kg P/ha on 11 Oct 2007.

The 18 clones of experiment 3 were grown on a similar basis to the four clones in experiment 1. One budded setts were planted in germination trays on 17 Jun 2009 and transplanted to 27-L pots on 27-31 July. Twelve of the 15 pots of each clone were moved to an area just outside the TPF on 14 Oct 2009. Eight pots of each clone were transferred to TPF chambers on 19 Oct 2009. Irrigation was applied to all pots sparingly to restrict plant extension rate to an average of about 2 mm/h in order to achieve high sucrose concentration based on experience gained in previous experiments where source-sink relationships were manipulated for this purpose (Inman-Bamber *et al.* 2008; 2009; 2010). Six 500 W lamps were mounted above the plants in each chamber and were timed to switch on at midnight for 1 h during January and February 2010 to suppress flowering.

Experiment 4 was conducted on the same pots and clones (8 pots per clone) used in experiment 3 after cutting regrowth back on 11 Oct 2010. Pots were irrigated automatically when soil water content determined with six frequency domain reflectometers (model CS615, Campbell Scientific Inc., (CSI) Logan, UT, USA), reached 34%. With this method of irrigation, plant extension was shown to proceed at maximum rates limited only by temperature (Inman-Bamber *et al.* 2008; 2010) so we could be certain that photosynthesis was not limited by water stress. Photosynthesis of leaf #1 was reduced only when plant extension rate declined below 50% of potential (Inman-Bamber *et al.* 2008).   Nutrients were applied in the form of 50 g Osmocote on 13 Oct and 22 Dec 2010.

Biomass sampling

In experiment 1 destructive sampling of four pots (replications) for each clone and irrigation treatment was carried out on four occasions, on 20-21 Dec 2006, 6–8 Feb, 20–22 Mar and 1–3 May 2007. Plants were cut at ground level and all the material weighed. Internodes were identified with reference to the leaf attached to the basal node so that the leaf with the youngest visible ligule (leaf #1) was attached at the base of internode #1. Internode #1 was included with the sheath material (cabbage) when leaf and stalk components were separated, weighed and dried to determine total above ground biomass per pot. Transverse segments (disks) ~5 mm thick were excised from the middle of internodes 2, 4, 6, 8, and then every third internode if they were present. The disks were quartered, placed in vials, and immediately frozen in liquid N_2 and stored at –80°C until sugars were extracted.

For experiment 2 in the field, sampling was undertaken on 20 Apr 2009 by removing 10 stalks at random from guard rows of each of the 20 selected clones, except F09 which could not be located with certainty because of lodging. Dead leaves, green leaves and sheaths from 5 of these stalks were separated, weighed, dried and weighed again. The 10 stalks in each sample were divided into tops (50 cm), butts (50 cm) and middle sections which were weighed separately. The length of the middle section of each stalk was recorded. Tops and butts (10 per sample) and mid sections (5 per sample) were fibrated, mixed and then analysed for sucrose, fibre and dry matter content following the methods of Muchow *et al.* (1993).

Experiment 3 was sampled three times in a manner similar to that of experiment 1, on 26-28 Oct 2009, 15-23 Mar and 19-28 Jul 2010. Samples for sugar analysis were taken from leaves 1 and 5 as well as from internodes 3, 5, 7, 10, 13 and 16. Samples for internodes #19 and older were thoroughly mixed to obtain average sugar concentrations for the base of the stalk.

Sugars (sucrose, glucose and fructose) were extracted from the frozen internode tissue kept at –80°C as described by Campbell *et al.* (1999). The sugar composition and concentration in the tissue samples were determined by HPAE-PAD as described by Papageorgiou *et al.* (1997). Glucose, fructose, and sucrose calibration curves (r^2 > 0.997) were produced from AR grade sugars (Sigma, St Louis, MO, USA). Sucrose content of dry matter of the internode tissue was derived from its sucrose and dry matter contents. Sucrose content of dry matter for internodes that were not sampled was obtained by interpolation, and sucrose content of dry matter for the whole stalk was the weighted mean sucrose content of dry matter of all internodes with internode dry mass as the weighting factor.

No biomass samples were taken in experiment 4.

Photosynthesis

Combined photosynthesis of all plants in two chambers of the TPF was measured on an hourly basis during experiments 1 and 3 following the method of Inman-Bamber *et al.* (2008). External air was passed through each chamber at a known rate and a sample of inlet and outlet air was passed simultaneously through the sample and reference tubes (differential mode) of an infrared gas analyser (IRGA, Li6262, Lincoln Nebraska). Net photosynthesis of all plants in each chamber was the product of volumetric airflow and the difference in $[CO_2]$ between inlet and outlet air.

Single leaf photosynthesis

A portable IRGA (Li6400, Lincoln Nebraska) was used to measure photosynthesis of individual leaves, usually leaf #1, in experiments 2, 3 and 4. Light (400-700 nm) intensity was set at 2000 $\mu mol/s.m^2$, the stomatal setting was appropriate for a 2:1 stomatal density on abaxial:adaxial surfaces, the leaf temperature was set to 27 to 32°C depending on ambient conditions, the CO_2 inflow concentration was set to 400 ppm and flow rate set to 300 to 500 mL/s in order to maintain VPD(leaf) <2.0 kPa. Stability was established when total CV% <1 in most cases but CV%<2 was used when power was low in experiment 2 in the field. In experiment 3 photosynthesis of leaves 1 and 5 was measured in Jul 2010 to investigate any possible feedback by sugars in internode #5 on its subtended leaf.   In experiment 4 we were concerned that leaves had time to adapt to the high light levels in the cuvette, particularly in the afternoon when solar radiation was low so the stability of CO_2 and H_2O fluxes was allowed to reach a maximum before taking a reading. Morning and afternoon readings were taken on days with limited cloud cover and compared in most cases with morning readings taken the next day. Large variation in photosynthesis was expected from the work of Irvine (1975) and so large numbers of readings were taken initially (Table 2) but the variation turned out to be smaller and fewer readings were necessary particularly in the controlled conditions of the TPF.

Table 2. Growth stages, dates and number of leaf photosynthesis measurements in experiments 2, 3 and 4. 1= before stalk elongation, 2 = stalk elongation phase, 3= harvest stage.

Experiment	Growth stage	Approx. date	Start date	End date	Leaf	Readings per clone	Total no of readings
2 – Field	1	Oct 08	2Oct 08	21Nov 08	1	25-45	673
2 – Field	2	Apr 09	27Mar 09	23Apr 09	1	26-38	632
2 – Field	3	Jun 09	2Jun 09	6Jun 09	1	19-20	390
3 – TPF	1	Oct 09	21Oct 09	30Oct 09	1	7-11	153
3 – TPF	2	Nov 09	26Nov 09	2Dec 09	1	8	144
3 – TPF	2	Apr 10	14Apr 10	16Apr 10	1	4-16	181
3 – TPF	3	Jul 10	7Jul 10	16Jul 10	1& 5	8-27	284
4 -outside pots	1/2	Jan 11	5 Jan 11	24 Jan 11	1	8-16	207

OUTPUTS

1. Results from field and glasshouse experiments

Experiment 1

Hourly photosynthesis per unit leaf area (A) of all well watered plants in experiment 1 responded to radiation (R) inside the glasshouse according to the function $A = Amax *(1-exp(-Q * R /Amax))$ where $Amax$ is photosynthesis at saturating light intensity and Q is the quantum efficiency or the slope of the curve at the compensation point. $Amax$ and Q both declined significantly (Fig. 3, Table 3) with crop age despite consistency in temperature and a favourable watering regime.
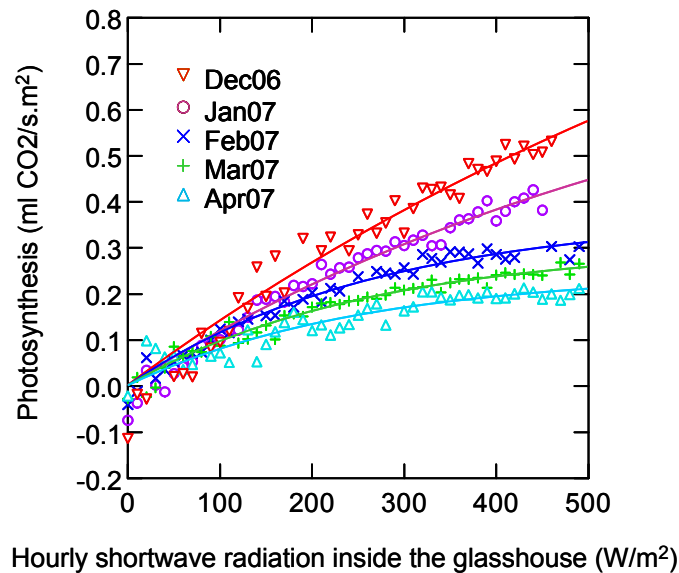

Fig. 3. Hourly photosynthesis for all well watered plants in response to radiation, grouped into months in experiment 1 (Inman-Bamber *et al.* 2009). Each point is the mean of five 12 min photosynthesis measurements and 60 radiation readings per hour. Lines were fitted as $A = Amax * (1-exp(-Q * R/ Amax))$ where A is net photosynthesis, Q is quantum efficiency and R is radiation.

Table 3. Estimates of photosynthesis at saturating radiation (*Amax*) and quantum efficiency (*Q*) for the data in Fig. 3 with lower and upper 95% confidence limits.

Month	Maximum photosynthesis (ml CO_2/s.m^2)			Quantum efficiency (ml CO_2/s.W)			N	r^2
	Estimate	Lower	Upper	Estimate	Lower	Upper		
Dec	1.961	0.907	3.016	0.00140	0.00129	0.00150	192	0.87
Jan	0.850	0.722	0.978	0.00129	0.00123	0.00135	708	0.85
Feb	0.389	0.355	0.423	0.00127	0.00120	0.00135	276	0.85
Mar	0.305	0.288	0.321	0.00116	0.00109	0.00122	696	0.71
Apr	0.229	0.214	0.243	0.00109	0.00100	0.00119	312	0.56

When hourly photosynthesis measurements were grouped into morning (9-10 h), midday (12-13 h) and afternoon sessions (15-16 h) and then regressed against hourly radiation (Fig. 4) using the model in Fig. 3, differences within each month (January and April) were significant for *Q* but not for *Amax* (Table 4). In January *Q* for the morning period was greater than for the midday period but it was not greater than for the afternoon period. In April, *Q* for the afternoon period was significantly greater than for morning and midday periods. The differences in the response of photosynthesis to radiation while significant were small and not consistent with inhibition by the accumulation of sugars in the leaf or stalk. Radiation in the TPF was about half the external radiation (Inman-Bamber *et al.* 2008) and it is possible that sucrose contents in leaves never reached levels high enough to feedback on photosynthesis. However diurnal feedback on photosynthesis is not being questioned or tested in this experiment since there is ample evidence that feedback does occur within the leaf (McCormick *et al.* 2009). Feedback from sucrose in the stalk would be expected to exacerbate this diurnal feedback from sucrose in the leaf causing reduced photosynthesis in the afternoon, more so as the crop developed. This was not observed in this experiment. Diurnal feedback resulting from high photosynthesis rates in the morning would be a constraint to biomass accumulation regardless of whether stalks had high levels of sugars or not.
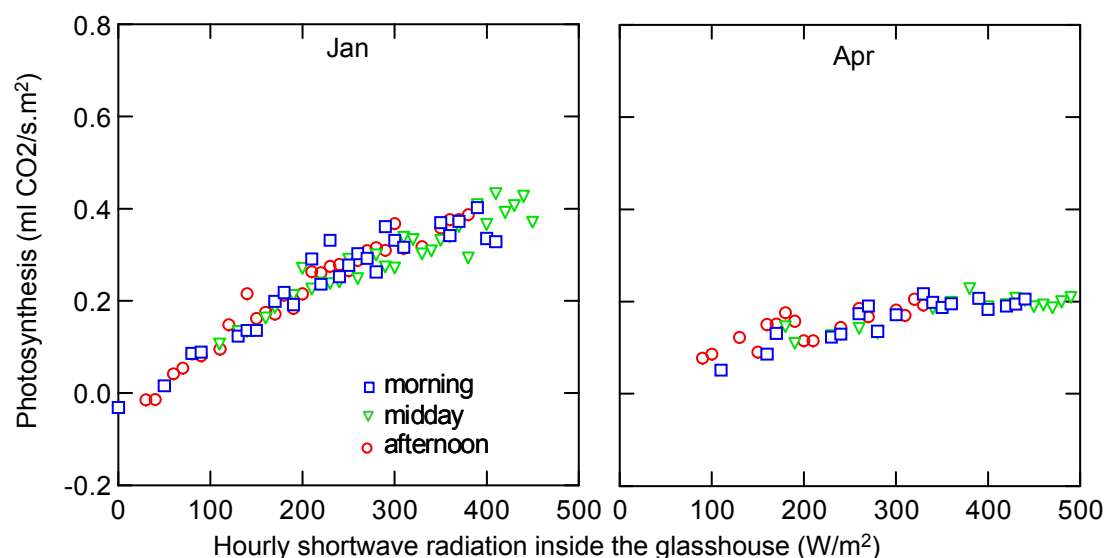
Fig. 4. Hourly photosynthesis of all well watered plants for January and April 2007 grouped into morning (9-10 h), midday (12-13 h) and afternoon sessions (15-16 h) in response to radiation in experiment 1.

Table 4. Estimates of photosynthesis at saturating radiation (*Amax*) and quantum efficiency (*Q*) for the data in Fig. 3 with lower and upper 95% confidence limits.

Month	Period	Maximum photosynthesis (ml CO_2/s.m^2)			Quantum efficiency (ml CO_2/W.s)			n	r^2
		Estimate	Lower	Upper	Estimate	Lower	Upper		
Jan	morning	0.748	0.547	0.948	0.00137	0.00125	0.00148	236	0.82
Jan	midday	1.291	0.651	1.931	0.00113	0.00103	0.00123	240	0.79
Jan	afternoon	1.404	0.655	2.153	0.00121	0.00112	0.00130	232	0.91
apr	morning	0.291	0.228	0.354	0.00085	0.00072	0.00098	104	0.68
apr	midday	0.278	0.226	0.330	0.00080	0.00067	0.00092	103	0.58
apr	afternoon	0.234	0.196	0.272	0.00124	0.00105	0.00143	104	0.53

Whole stalk sucrose concentration on a dry weight basis increased from < 0.2 in December 2006 to nearly 0.5 g/g in the high sucrose clones in May 2007 (Inman-Bamber *et al.* 2008) which is what can be expected in mature commercial cane crops after optimum drying off (Inman-Bamber 2004). Photosynthesis decreased as sucrose increased possibly as a result of feedback inhibition as sucrose content reached some limit or trigger point. However when the sucrose profile down the stalk was considered it was clear that while sucrose accumulated in internodes at the base of the stalk, sucrose content in the distal internodes remained low regardless of whether clones were of the high or low sucrose content category (Fig. 5).
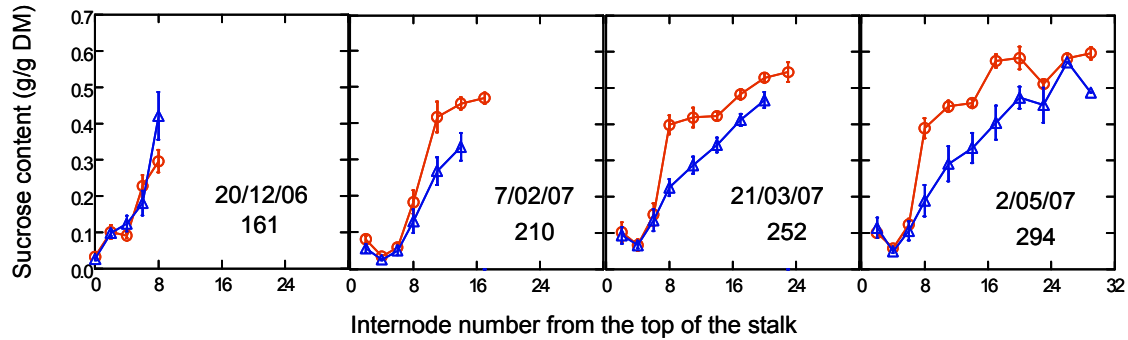
Fig. 5. Mean sucrose content of internodes of well watered plants of high (○) and low (△) sucrose content clones for four dates and days after planting in experiment 1. Bars are 2 x standard errors.

Experiment 2

Highly significant differences between clones in photosynthesis rates of leaf #1 were evident during all three rounds (phases) of measurements (Fig. 6). Variation in photosynthesis was greater amongst the high fibre group than the high sucrose group possibly because the latter included seven elite cultivars while the former were all unselected clones. Photosynthesis declined with age but this decline varied between clones substantially such that photosynthesis for F07 was only 25% lower in Jun 2009 than in Oct 2008 whereas photosynthesis for F01 was 70% lower. Clones in the high sucrose group varied less than those in the high fibre group in this regard. Photosynthesis was reduced significantly with lodging and flowering and these effects appeared to be additive.
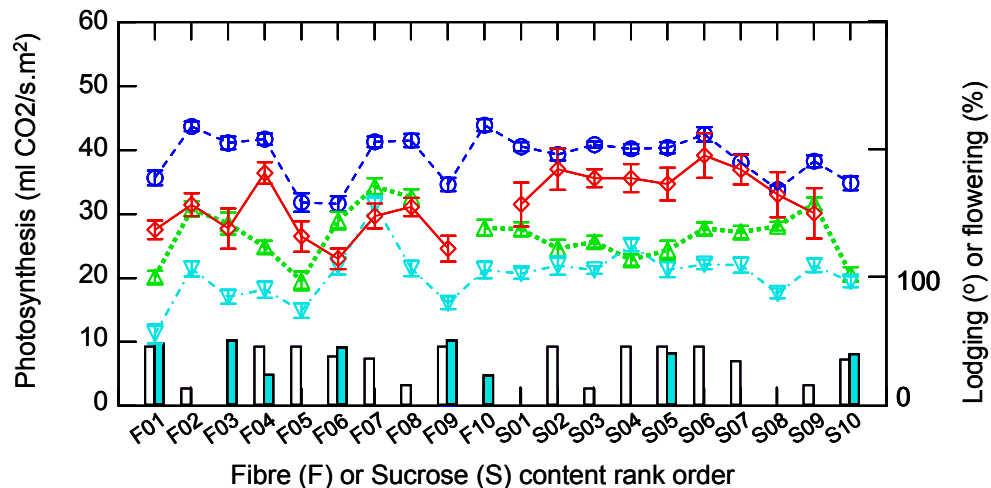
Fig. 6. Photosynthesis means for three growth stages in experiment 2; stage 1 (○- - -), 2 (△····) and 3 (▽·–·–·) and growth stage 1 (◇——) in experiment 3 (see Table 3), for 20 clones. The X-axis shows clones in order of sucrose or fibre content, F01 = highest fibre content, S10= lowest sucrose content determined before this project. Vertical bars along x-axis are ratings for lodging degree from vertical (solid) and % flowered stalks (open). Thin bars around photosynthesis means are 2 x standard error of the mean.

If the accumulation of sucrose in stalks of high sucrose clones was limiting photosynthesis through feedback inhibition, we would expect the reduction in photosynthesis over time to be greater in the high sucrose than the high fibre group of clones. This was not the case regardless of whether this change was expressed as an absolute difference or a relative difference (Table 5). On the contrary the decline in photosynthesis between Apr 2009 and Jun 2009 was greater for the fibre group than for the sucrose group (Table 5). Other differences were not significant.

Table 5. T-test statistics for absolute (abs) and % reduction in photosynthesis between growth stages 1, 2 and 3 for the high fibre and high sucrose groups of clones and cultivars.

Growth stage		Mean change		n		P
		High fibre	High sucrose	High fibre	High sucrose	
1&2	abs	11.0	12.5	9	10	0.43
1&2	%	72.2	68.1	9	10	0.42
1&3	abs	19.2	17.5	10	10	0.39
1&3	%	50.6	55.1	10	10	0.32
2&3	abs	8.3	5.0	9	10	0.04
2&3	%	70.0	82.3	9	10	0.03

Experiment 3- Photosynthesis

Leaf #1 photosynthesis in Oct 2009 varied significantly between clones (Fig. 6) and was less variable for sucrose types than for fibre types as noted in the field with experiment 2. Photosynthesis was lower for potted plants outside the TPF than for plants in the field even though plants were at a similar age at the same time of year (Fig. 6). Photosynthesis measurements taken in the field in Oct 2008 and those taken in potted plants in Oct 2009 were correlated significantly ($r^2 = 0.37$, p<0.01) but this was due to more to the variation in photosynthesis of fibre types ($r^2 = 0.62$, p=0.01) than in sucrose types. These early measurements of photosynthesis taken before the stalk elongation phase could be used as a baseline for later measurements.

The natural variation in photosynthesis between clones could mask any direct effects of sucrose on photosynthesis but if the reduction in photosynthesis with age is consistently greater in high than in low

sucrose clones this could add to evidence that sucrose accumulation in the stalk does practically inhibit photosynthesis and that, intrinsically, higher biomass could be achieved by selecting for low sucrose and high fibre. Photosynthesis was thus expressed relative to the baseline measurements made before the stalk elongation phase (Fig. 6).

Photosynthesis of one of the fibre clones (F06) was low in Oct 2008 and Oct 2009 (Fig. 6) but F06 was distinctive in retaining this level of photosynthesis throughout experiment 3 (Fig. 7a). Two other fibre clones (F04 and F08) were distinguished by a rapid decline in photosynthesis after Oct 2009 when photosynthesis was particularly high in F04 but not F08 (Fig. 7). The variation in the decline in photosynthesis was lower for the sucrose clones than for the fibre clones, however S07 and S09 maintained photosynthesis at significantly higher rates than S02, S04 and S08 for example (Fig. 7b). Mean photosynthesis for fibre and sucrose clones declined equally over the 10 month growth period so that photosynthesis was about 40% lower in both types in July 2010 compared to mean baseline photosynthesis in Oct 2008 and Oct 2009.
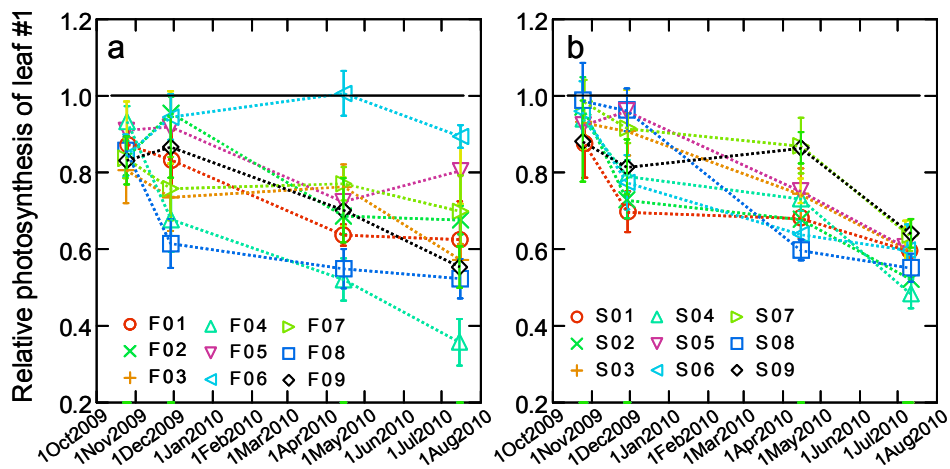
Fig. 7. Photosynthesis of leaf #1 relative to measurements taken before stalk elongation in Oct 2008 and Oct 2009 for nine fibre clones (a) and for nine sucrose clones (b). Bars are 2 x standard errors.

Relative rates of photosynthesis (April/October) for the field experiment (experiment 2) and the TPF experiment (April/October) were correlated (r^2 = 0.28; p=0.03, n=17) suggesting that maintenance of photosynthesis over time is determined genetically.

Experiment 3 - Sucrose content and photosynthesis

On a fresh weight basis, mean sucrose content for high fibre and high sucrose clone groups increased with internode age as expected and reached a high level in basal internodes of sucrose clones (>17%) but not in fibre clones (~10%) (Fig. 8). Mean sucrose content differed greatly between the fibre and sucrose groups of clones as expected but the differences were significant only for internode 7 and older (Fig. 8).
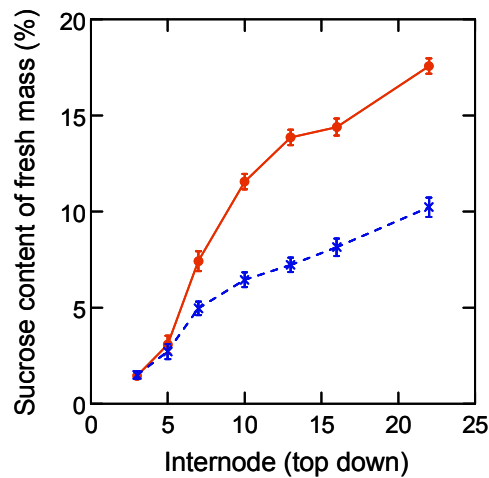
Fig. 8. Mean sucrose content of fresh mass for internodes of nine fibre clones (broken line) and nine sucrose clones (solid line) sampled at 11 months of age in July 2010. Bars are 2 x standard errors.

Individual clones differed significantly for sucrose content in all internode groups shown in Fig. 9 with large differences evident between fibre and sucrose clone types in the groups with internodes 7 and older. However the difference in sucrose content between sucrose and fibre types was indistinct for internodes 3 and 5 with some fibre types containing more sucrose than some sucrose types. Thus while sucrose storage in sucrose clones may have reduced sink strength for photo-assimilate as maximum levels were approached there was no evidence that storage capacity was limiting in the upper internodes or that this capacity was greater for fibre clones than for sucrose clones.
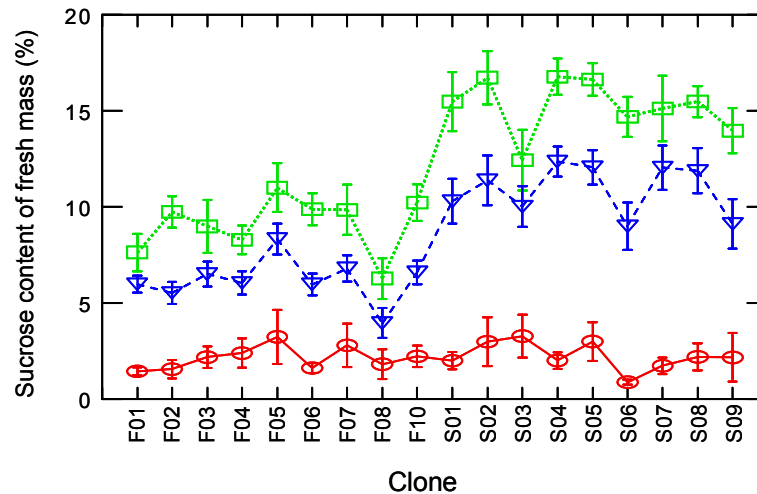
Fig. 9. Mean sucrose content of fresh mass for nine fibre and nine sucrose clones sampled at 11 months of age in July 2010 for internodes 3 & 5 (○——), 7, 10 & 11 (▽---) and 16 and the remainder of the *stalk* (□····). Bars are 2 x standard errors.

Photosynthesis for leaf #5 was not correlated with sugar content of internode #5 whether expressed on a fresh mass basis or dry mass basis (data not shown). Photosynthesis was not correlated to sugar content of any internode or leaf apart from near significant correlations (p<0.08) with sucrose content in leaf #5 and mean glucose content of internodes 3 to 16 (data not shown). In both cases the association between photosynthesis and sugar content was positive rather than the negative association expected if sugars were inhibiting photosynthesis.

Experiment 3 – Biomass and photosynthesis

Biomass yield of F08 was low (Fig. 10), consistent with a rapid decline of photosynthesis (Fig. 7). Biomass yield of F06 ranked in the top three of the fibre group in March and in July, which is consistent with its sustained photosynthesis although this was not particularly high to start with. Biomass yields of fibre types varied substantially in the range of 1 to 3 kg/pot at the final harvest and biomass yield of sucrose types was less variable, in the 2.3 to 3 kg/pot range (Fig. 10). This is consistent with the large variation in initial and subsequent photosynthesis for fibre clones compared to the narrow variation in both initial and subsequent photosynthesis for sucrose types (Fig. 6). However biomass was not correlated with leaf #1 photosynthesis at any stage other than when photosynthesis was determined in Oct 2009. In this case biomass in Oct 2009 was negatively correlated with photosynthesis (r^2 = 0.5, p = 0.001, n=18) possibly reflecting an early decline in photosynthesis with clones that were developing more rapidly than others. Photosynthesis for some clones declined rapidly between Oct and Nov 2009 (Fig. 7) but these were not necessarily the high yielding ones.

Biomass % gain from March to July differed significantly between clones (60 to 147%, p=0.04) but not between means for fibre and sucrose clone types (100 and 102%, p=0.90). The large difference in sucrose content between these groups thus appeared to have little effect on biomass accumulation in the later stages of growth.
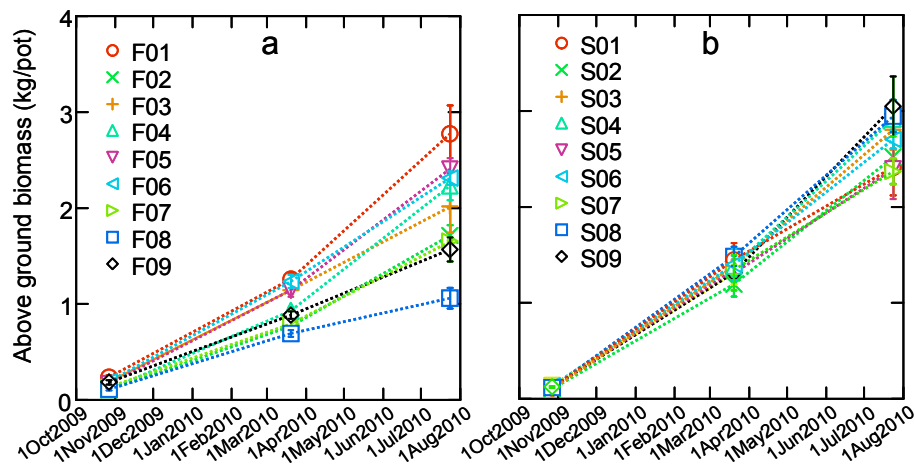
Fig. 10. Mean above ground biomass per pot for nine high fibre clones (a) and nine high sucrose clones (b) sampled on three occasions. Bars are 2 x standard errors.

Experiment 4

Clone mean afternoon photosynthesis was 9 to 36% (mean 21%) lower (p<0.001) than clone mean morning photosynthesis (Fig. 11). Photosynthesis of clones differed significantly (P<0.001) but the interaction between clones and time of day was not significant. Mean morning photosynthesis for each clone in this experiment was highly correlated (r^2 = 0.66) with mean photosynthesis for the same clones determined at about the same growth stage in the field for experiment 2 indicating that photosynthesis for leaf 1# is genetically determined to a large extent provided photosynthesis is measured under non limiting conditions of soil moisture, N, radiation, temperature and CO_2 concentration as was intended for experiments 1 and 4. Fibre clones F07 and F02 had particularly high rates of photosynthesis in experiment 4 (~45 µmol/s.m^2) but these did not lead to high levels of biomass amongst the fibre clones in experiment 3 (Fig. 10a).

Fig. 11. Morning (9:00-11:30, ●——) and afternoon (3.30 – 5:00, X····) and afternoon/morning (□---) photosynthesis of leaf #1 during the early stalk elongation phase (Jan 2011) for 18 clones grown in pots outside the TPF. Bars are 2 x standard errors.
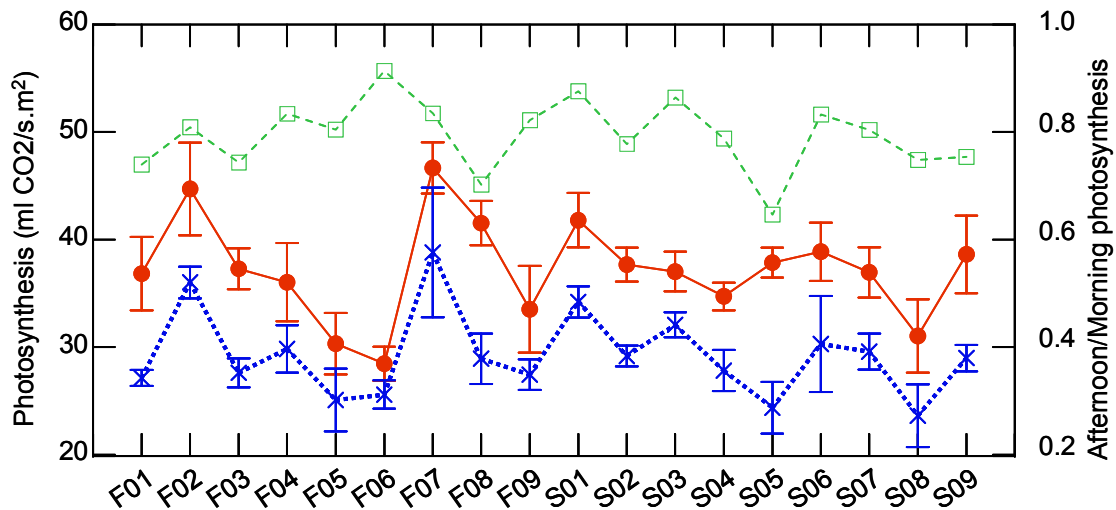
2. Conclusions from field and glasshouse experiments

If and when biomass from sugarcane becomes a priority for renewable energy production, our data suggest that photosynthesis is not suppressed when sucrose content in cane stalks reaches some critical level and so clones with a high fibre content do not have an inherent advantage over clones with high sucrose content. Commercial sugarcane varieties are subjected to intense selection pressure for high sucrose content and high cane yield in Australia (Jackson 2005) and these exhibited very high rates of photosynthesis of single young leaves, at least prior to stalk elongation. Rates exceeding 40 μmol/s.m^2 were found in several high sucrose types and even in some unselected high fibre types at this growth stage. However leaf #1 photosynthesis was not correlated with biomass accumulation despite the large variation in both variables. Irvine (1975) measured leaf photosynthesis in two groups of 30 clones of *Saccharum* spp exhibiting a wide range of yield and other traits but he found no correlation between photosynthesis and any expression of yield. By contrast whole plant photosynthesis and biomass accumulation in sugarcane were found to be highly correlated (Inman-Bamber *et al.* 2009) as one would expect. Thus the focus on single young leaf photosynthesis in much of the published work on feedback inhibition of photosynthesis by sucrose (van Heerden *et al.* 2010) may be not be closely related to whole plant and whole crop effects because other leaves and the total leaf area may have overriding effects on whole plant photosynthesis and yield. Leaf area index was proposed as a better

indication of yield after Irvine (1975) failed to find a correlation between single leaf photosynthesis and yield.

Photosynthesis of whole plants and of single leaves decreased with crop development as much as 60% in some cases. While the decline in photosynthesis with crop age and with leaf N content has been known for some time (Kortschak and Forbes, 1969 as cited by Alexander 1973) we are not aware of similar reports for a decline in whole plant photosynthesis with crop age. Some clones in our experiments maintained high rates of leaf #1 photosynthesis for longer and variation for maintenance of photosynthesis was greater in the fibre group than the sucrose group of clones. Maintenance of high photosynthesis rates was not associated with low content of sugars in leaves or in internodes. While sucrose contents in most internodes were considerably greater in high sucrose than in high fibre clones the sucrose content of upper internodes (3 and 5) was higher for some fibre clones than for some sucrose clones. In experiment 1 the sucrose content on a dry matter basis of internodes 6 and younger was <0.15 g/g with a capacity to increase to 0.60 g/g as for basal internodes (Fig. 5). The sucrose content of young internodes increased substantially when their sink strength was limited through the reduced elongation rate imposed by limited watering (Inman-Bamber *et al.* 2009). We argue that if sink strength was limiting photosynthesis in experiment 3 the sucrose content of the upper internodes would have been much higher particularly for the high sucrose clones. This was not the case.

McCormick *et al.* (2009) cited research by Irvine (1975) as an example where co-ordination between leaf photosynthesis and stalk (culm) sink strength resulted in higher rates of photosynthesis in wild types of sugarcane (*Saccharum spontaneum*) than in high sucrose hybrid or noble canes (*S. officinarum*). Irvine (1975) did not reach this conclusion from his data which indicated that the wild types had a much larger range in photosynthesis than the commercial clones because of the much narrower genetic base in the commercial clones. Our results agree with those of Irvine (1975) in this regard but do not support the association between stalk sucrose content and photosynthesis proposed by McCormick *et al.* 2009) who also associated reduced photosynthesis with maturity (high sucrose) noted in other early publications (eg Allison *et al.* 1997).   Our results showed substantially reduced photosynthesis of whole plants and of single leaves with crop age and marked increases in sucrose content were observed at least in the first experiment (Inman-Bamber *et al.* 2008) and could be assumed for the others. However our data suggest that while photosynthesis and sucrose content of the stalk change with crop age, sucrose accumulation in the stalk is not the basis for reduced photosynthesis. Allison *et al.* (1997) linked the age reduction in photosynthesis to reduced specific leaf N (SLN) rather than to feedback inhibition from sucrose but they do not offer an explanation for the

decline in SLN despite regular application of N in the nutrient solution. Rather they suggest searching for variation in the uptake of N later in the crop cycle in order to maintain SLN at high levels for longer.

Photosynthesis of leaves of sugarcane declined in the afternoon after rapid increases during the morning in sucrose and hexoses (McCormick *et al.* 2008). When leaves were kept in the dark in the morning and then transferred to the light, photosynthesis was higher than it was in the morning and considerably higher than afternoon photosynthesis of leaves exposed to full sunlight all day (McCormick *et al.* 2008). This and other research by McCormick *et al.* (2006; 2009) confirm that sucrose or hexose levels in the leaf suppress photosynthesis in the afternoon or at anytime when these sugars are at high levels due to restricted translocation or artificial feeding. The 9 to 36 % reduction in photosynthesis in the afternoon found in experiment 4 is consistent with the observations of McCormick *et al.* (2008; 2009). Plants in experiment 4 were young with little stalk material and levels of sucrose in the internodes were probably very low based on the data from previous experiments (Fig. 5). We therefore suggest that the build up sugars in the leaf and consequent reduction in photosynthesis has little to do with sucrose accumulation in the stalk. As our results and those of others (Amaya *et al*, 1995) show, the highest rates of photosynthesis are found in young leaves of young plants and one would expect localized feed back to be stronger in these circumstances than in young leaves of older plants or in old leaves of young or old plants (Vu *et al.* 2006). Most of the research on feedback inhibition of photosynthesis is based on photosynthesis of segments (6 - 100 cm^2) of single leaves (McCormick *et al.* 2006; 2008). Our results on photosynthesis of whole plants indicate that there is little change through the day in the response of photosynthesis to light regardless of plant age. Radiation levels in the TPF were about 55 % of full sunlight (Inman-Bamber *et al.* 2008) but maximum rates of photosynthesis were not much lower than single leaf measurements in the morning in the experiments by McCormick *et al.* (2008). However the mean sucrose content of leaves #1 and #5 of 13 month old plants determined throughout the entire day in experiment 3 was similar to the sucrose content measured by McCormick *et al.* (2008) at 9 am. Localised feedback in young leaves may be compensated by more rapid photosynthesis in older leaves. Photosynthesis of the 3rd youngest fully expanded leaf increased 48% when all other leaves were shaded for 14 d (McCormick *et al.* 2006) indicating the extent to which older leaves can respond if photosynthesis in younger leaves is inhibited for some reason.

Our data support previous conclusions about localised feedback on photosynthesis by sugars accumulating in the leaf resulting in reduced photosynthesis of small segments of individual young leaves. However whole plant photosynthesis did not decline through the day indicating that older leaves may compensate for reduced photosynthesis in younger leaves in the afternoon. While the sucrose

content of young internodes can increase substantially when their sink strength is limited, the sucrose content of young internodes remained low in both high fibre and high sucrose clones leading to the conclusion that 'storage capacity' did not limit photosynthesis at any stage of development.   While photosynthesis declined with crop age and sucrose content increased we found no evidence to suggest that photosynthesis declined *because* sucrose content increased. While there is considerable variation in photosynthetic capacity of young leaves in the Australian genepool, there was no indication that this trait is linked to biomass yield in a simple way. Variation also exists in the rate at which photosynthesis of young leaves declines with crop age. It is likely that biomass yields will increase if biomass is the target trait for selection rather than sucrose yield but increased biomass yield may not necessarily result in reduced sucrose content and increased fibre content. Photosynthesis of whole plants is well linked to biomass accumulation but it would be easier to simply select for biomass or high sustained radiation use efficiency rather than whole plant photosynthesis.

3. Model for estimating biomass yields in marginal areas

Feedback on photosynthesis by sucrose accumulation in the stalk was shown to be unimportant in the later growth stages of sugarcane (Inman-Bamber *et al.* 2011 – Appendix 1) and growth slow down reported by Park *et al.* (2005) and van Heerden *et al.* (2010) has yet to be explained physiologically in situations where lodging was not observed. Van Heerden *et al.* (2010) raised the possibility that respiration of labile sugars in the stalk could possibly explain the growth slow down phenomenon (RGP). RGP was most noticeable during growth analysis experiments in the Ord River Irrigation Area (ORIA) where the crop was expected to accumulate large amounts biomass but failed to do so (Fig. 12). These expectations were based on the APSIM-Sugarcane model which was developed for the sugarcane crop in the east of Australia. The model has been validated extensively in other countries as well (Keating *et al.* 1999). Large adjustments were required to the default APSIM settings to represent the Ord crop more accurately. These adjustments were a decrease in radiation use efficiency (RUE) when more than 30 t/ha biomass had accumulated and an increase in transpiration efficiency (TE) from 8 to 10 g kPa/kg for the duration of crop development. The RUE reduction was from 1.8 to 1.0 g/MJ in plant crops and from 1.65 to 1.0 g/MJ in ratoon crops. The effect of these substantial adjustments on yield simulations is shown in Fig. 12 (Inman-Bamber *et al.* 2006).
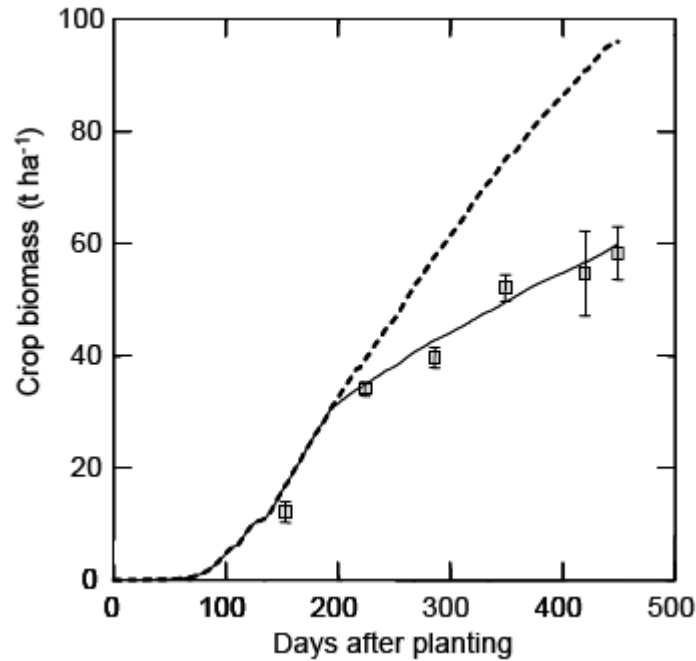
 Fig. 12. Measured and simulated crop biomass with (solid line) and without (broken line) adjustments to the APSIM model (Inman-Bamber *et al.* 2006)

WaterSense is a web based model developed for irrigators in the Ord as well as in the east where it is now used particularly in the southern (Bundaberg) and northern (Atherton) regions of the industry (Haines and Attard 2010). WaterSense incorporated the best appropriate components of APSIM and CANEGRO for use in irrigation scheduling over the web (Inman-Bamber *et al.* 2007). A model called WSworkbench was used as a testing platform during the development of WaterSense and is essentially the same as WaterSense but can be used to test new components such as the response of sugarcane high temperature and it can be used to assess new production systems such a biomass production system for marginal areas. WSworkbench is not user-friendly but it is quick and very flexible.

Three new growth analysis experiments (unpublished) were conducted in the Ord from 2003 to 2006 in conjunction with Bowen ratio measurements of crop water use. These data can be used to help explain the typical growth slow down as observed in Fig. 12, using WSworkbench.

Field 9a was ratooned after harvesting a 2nd ratoon of Q99 on 17Sep03 and the 3rd ratoon (9a03) was sampled periodically for biomass and other yield components. A plant crop of Q95 in field 9d was harvested on 2Sep04 to allow for serial harvesting of the 1st ratoon (9d04). A third experiment (11d05) was conducted on a 1st ratoon of Q99 harvested very early in the season on 5May06 to test the 30 t/ha biomass yield threshold for growth slowdown.

An equation [R = S(0.15exp(0.07Tmean)) g/m^2/d] for respiration (R) to estimate the respiration of sucrose (S) was obtained from Liu and Bull (2001) and was calibrated to the sucrose content data from these three experiments (Fig. 13).

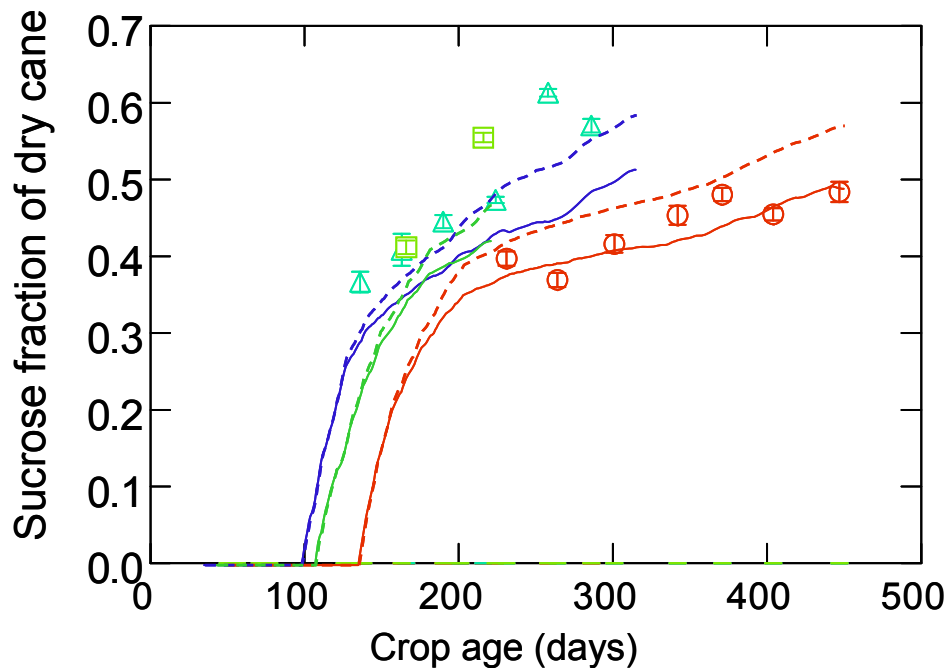Fig. 13. Measured (symbols) and simulated sucrose content on a dry matter basis, with (solid lines) and without (broken lines) sucrose respiration using the WSworkbench model.

It seems from Fig. 14 that respiration of sucrose could account for the slow down of growth in high temperature conditions since this was the only 'adjustment' required to simulate biomass yield for the three experiments, using WSworkbench.
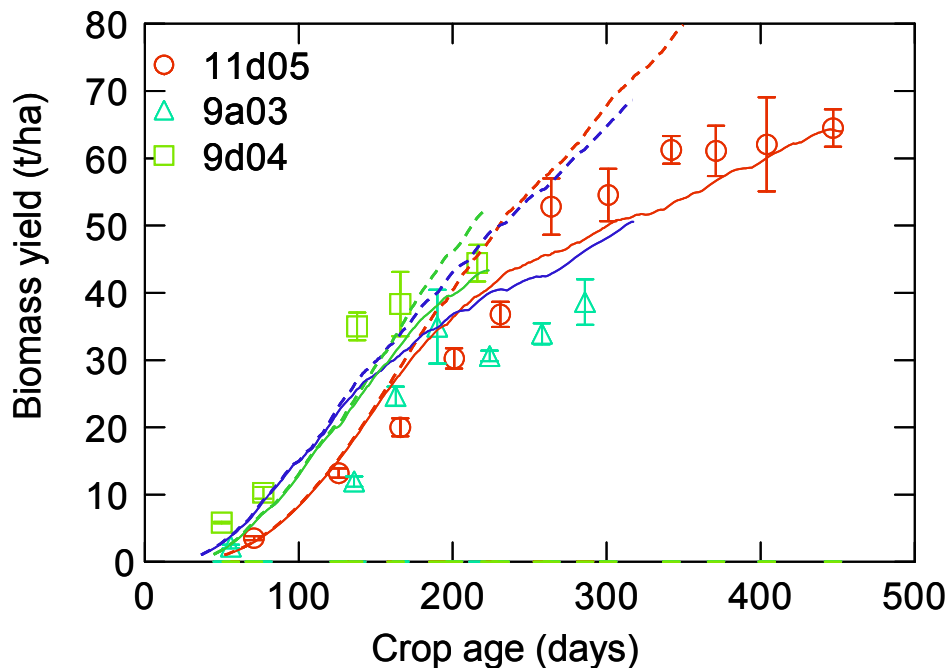
Fig. 14. Measured (symbols) and simulated crop biomass with (solid lines) and without (broken lines) sucrose respiration using the WSworkbench model.

4. Estimates of potential biomass yields in marginal rainfed conditions

If sugarcane is to be used as a feedstock for a biofuel industry it will probably need to be grown in marginal areas with limited or no irrigation making use of rainfall when it occurs particularly in the wet season. An option to simulate such a production system was added to WSworkbench which started the crop on a given date and then allowed climatic conditions to govern the harvest and ratooning cycle. The model allowed the crop to be harvested 14 days after all the available soil water had been extracted and before large biomass losses occurred due to respiration and stalk death. The stool was assumed to remain alive and able to grow normally when more than 50 mm of rain was received. If rainfall conditions allowed, the crop was harvested on a designated date for each paddock, provided it had produced more than 5 t/ha biomass. If this yield had not been reached by the given harvest date the crop would be allowed to continue growing for another year or until the soil dried out completely for 14 days.

Other conditions of the simulations were:

1) The soil type was a favourable Red Ferrosol with a plant available water capacity of 184 mm (Inman-Bamber *et al.,* 2000).

2) Silo climate records were from 1960 to 2011 for Mareeba, Bambaroo, Millaroo, Charters Towers, Bowen, Eton (Mackay) and Bundaberg (Airport).

3) Silo data do not include wind speed and an average of 2 m/s was assumed for this.

4) The crop coefficient for transpiration was 1.2 up to 30 t/ha biomass and it tended to 1.0 at a biomass yield of 80 t/ha.

5) Nominal ratoon/harvest dates were 45 days apart to span a typical crushing season; 13 June, 28 July, 11 September and 26 October.

6) The crop could regenerate after more than 50 mm rain fell in one day.

7) The dry day count was restarted when soil water content exceeded 20 mm (11%).

An example of a late season cycle starting on 26 October in 1960 and then following the above rules for harvesting and ratooning till 2011 is shown in Fig. 15. The simulation assumed that the stool would remain alive after harvesting in dry soil but in practice replanting would be required either because of stool death in conditions that remain dry or after a limited number of ratoons even if the rain occurs frequently enough to keep the stool alive. We do not know how long stools can survive in dry conditions so the requirements for replanting are difficult to model at this stage. Stool survival is probably linked to the extent to which genes of the clone derive from *S. spontaneum* parentage and it is likely that 'biomass' clones will come from crosses with more of these genes than are present in our commercial hybrids currently.

No crops could be harvested at the target harvest age (12 months) at Mareeba, Millaroo, Charters Towers and Bowen, all succumbing to severe soil water deficits after the wet season (Fig. 15). Some crops in the more southerly sites (Eton and Bundaberg) were older than 12 months when harvested because they ratooned from crops that were harvested prematurely and then received enough rain to germinate   before   the   nominal   ratoon   date   (26   October).
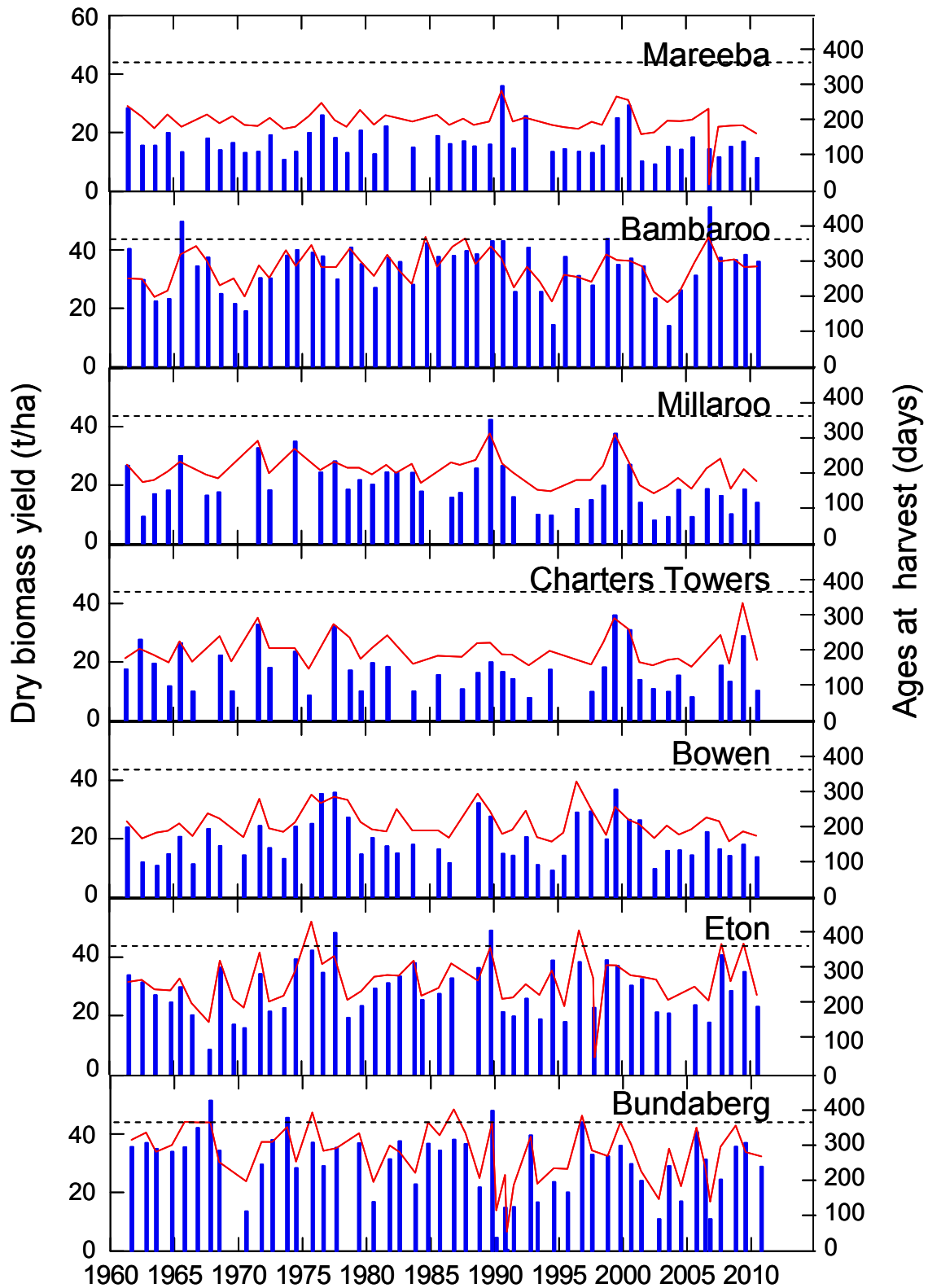
Fig. 15. Simulated biomass yield (bars) for a nominal late season crop (26 Oct) harvested and ratooned according to the rules described in the text. Actual age at harvest   (solid red line) and target age (broken line).

Bambaroo was the most favourable site of the seven marginal sites considered, producing a reasonable crop every year and a mean of 34 t biomass /ha over 50 crops (Fig. 16). Mareeba was the least favourable site producing only 17 t/ha biomass per year on average and not producing any harvestable biomass in 3 to 4 out of the 50 years in the simulation. Mean yields at Millaroo and Charters Towers were slightly better but the variability was greater with up to 11 failed crops in the 50 year period (Fig. 16). Biomass yields tended to be higher with earlier starting dates but the variability in biomass yield from year to year indicated that nominal starting date was less influential than climate variability in determining performance.
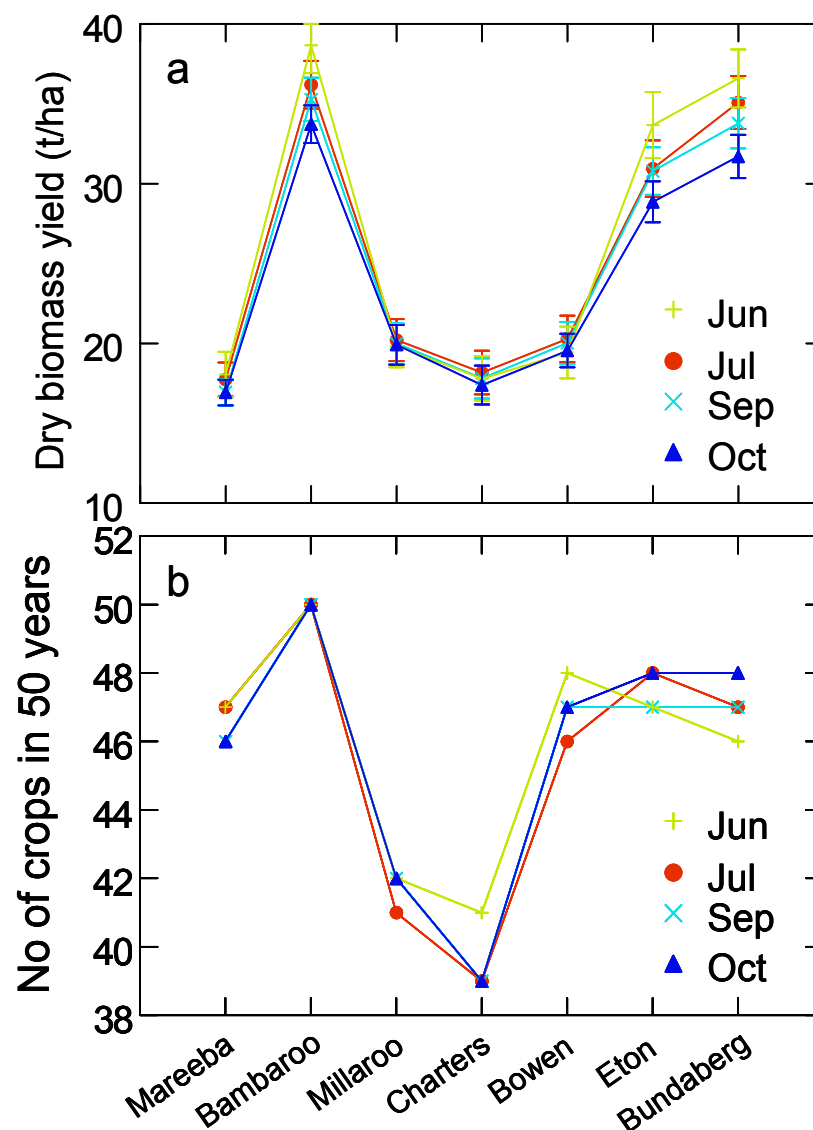
Fig. 16. Mean biomass yield per annum (a) and number of crops in 50 years (b) for seven 'marginal' sites where sugarcane may be grown for biomass without irrigation for crops starting nominally on 13 June, 28 July, 11 September and 26 October each year. Bars are 2 x standard errors of the mean.

The production system envisaged for biomass production was one that made use of rainfall only and where stools could survive for several months without rain and be capable of regeneration once rains started again. No doubt some breeding and selection will be required for such a system and this modelling will help to define the conditions under which such selection should occur. Large variability in rainfall would result in large variability in biomass production over time and this would have to be factored in the economics of producing and processing biomass from sugarcane. In some areas farmers would have to accommodate the possibility of having no biomass production in some years and possibly no yield two years in succession.

5. Benchmarks for sucrose content and photosynthesis (Appendix 2)

Prior to project CSE023, the maximum recorded sucrose content obtained in a reliable way (HPLC) was reported by Berding (1997) while examining 154 unselected clones in a selection trial at Mulgrave, Australia. The juice from whole sound stalks contained 23.5% sucrose and sucrose was 60% of stalk dry matter for one clone (2 replications). This remains as the record for sucrose content of whole sound stalks. Sucrose was 58% of stalk dry matter for another 11 clones in this trial. Prior to our work on sucrose accumulation (CSE014 and CSE023) the highest sucrose content of fresh cane stalks (20.5 %) was reported by Venkataramana and Naidu (1993) for basal internodes of Co7704. These authors also measured very high sucrose in the top three (distal) internodes (18.9%) but no one else has published values anywhere near this for internodes at the top of the stalk. It is not clear what was meant by the top three internodes in the paper by Venkataramana and Naidu (1993) and it is possible that they topped the stalk much lower than at the apical meristem (at the base of the youngest mature leaf) which is the accepted 'top' in more recent publications. In this project (CSE023) sucrose content in the top 5 internodes was always low and subject more to variation in the environment than to genotypic variation even with the wide range of clones used. Sucrose content determined by HPLC reached 21% of fresh weight and 67% of dry weight in basal internodes of Q117 where expansive growth had been constrained by water stress in the TPF (Inman-Bamber *et al.* 2011).

Wu and Birch (2007) published a record 27% for sucrose content plus its isomer isomaltulose in genetically modified sugarcane plants.

Dry matter content in commercial sugarcane cultivars was as high as 37% after a long and moderate dry off period Inman-Bamber (2004) but Wang *et al.* (2008) reported dry matter contents up to 41% in crosses between *S. officinarum* and *S. spontaneum*. These clones also presented very high fibre

contents of up to 29% on a fresh mass basis. In CSE023 our maximum dry matter content was 41 % for a basal internode of a high fibre clone.

Maximum photosynthesis rates for sugarcane have been reported under elevated CO_2 conditions and for plants which have been tampered with in some way. All high rates (up to 43.5 µmol $CO_2/m^2.s$) were for small segments (2-10 cm^2) of young leaves, normally the youngest mature leaf. IN CSE023 high fibre clones QB01-5 and CT04-495 had mean photosynthesis rates of 44 and 46 µmol $CO_2/m^2.s$ for leaf #1 measured between 9 and 11 am, before stalk the elongation phase. Afternoon readings were lower.

 Photosynthesis on a m^2 leaf area basis is expected to be considerably lower for whole plants than for leaf segments and ours is the only recent work reporting such a measure reaching 17 µmol $CO_2/m^2.s$ (Inman-Bamber *et al.*, 2008) at a light intensity of 1200 µmol $CO_2/m^2.s$ and 18.6 µmol $CO_2/m^2.s$ at light saturation.

Transpiration efficiency (TE, ratio of photosynthesis to transpiration) has seldom been reported for sugarcane and a benchmark value of 19.6 g CO_2/Kg water was found for plants growing in 2 x ambient CO_2 concentration. The nine high sucrose clones used in CSE023 had a median TE of 17.4 and 9th decile of 27.2 g CO_2/Kg water for leaf 1, prior to rapid stalk elongation.

The benchmark TE in terms of aerial biomass accumulation (8.7 g dry mass/Kg water at 1 kPa vapour pressure deficit) was established in our work also funded by SRDC (Inman-Bamber and McGlinchey, 2003). This value is higher than the previous benchmark (8.0 g dry mass/Kg water) assumed by Keating *et al.* (1999) for use in the APSIM-Sugarcane model.

6. Options for increasing CCS (Appendix 3)

The maximum CCS in commercial cane supplied to mills currently is about 18% which is close to the maximum sucrose content of internode tissue (21%) measured when carefully controlling expansive growth to enhance sucrose content. While a small fraction of consignments are near to this observed upper level, the industrial average has fluctuated between 13 and 14% without increase for many years. This indicates that most industry cane consignments are not close to the biological upper level. The shortfall of 4 to 5% units between consignments with the highest CCS and the industry average arises due to two reasons. Firstly, it is due to responses of current cultivars to environmental factors acting to

divert assimilate to growth rather than storage of sucrose, rather than an inherent upper bio-physical limit already attained in these cultivars. This is particularly so for cane harvested outside the time of the year when CCS is highest. Consequently, there is a further opportunity to both manage crops and select varieties that have higher CCS earlier in the season. However, the apparent slow general progress in breeding programs and agronomic research in leading to industry outcomes justifies a re-think of past approaches.   Results from recent physiological experiments point to source/sink balances as being important for maximising CCS.   There is some evidence suggesting high CCS genotypes may be sensitive to factors affecting stem elongation. Moderate reductions in expansive growth that reduce cane volume and weight without sacrificing photosynthesis lead to improved CCS. Re-evaluation of ripeners with a better understanding of ripener, water, temperature and genotype interactions possibly using controlled conditions may be useful.   Secondly, industry CCS is reduced by extraneous matter, as is well known. This is an issue related to economic optimisation of harvester design and operation, variety selection and milling season.   Current biases in predicting economic value of varieties in breeding programs arises because sound whole stalks are used as a measure of variety CCS and this may be inhibiting the rate of genetic gain in mill CCS to the extent that there is important genetic variation in suckering and in other traits affecting extraneous matter. The final way of increasing CCS would be to raise the potential CCS to get beyond the measured limit (21%). This will require exploiting genes that are not yet in the commercial germplasm pool and molecular intervention to break internal physiological limits where the highest reported concentration of sugars in transgenic plants is about 27% on a fresh weight basis (Wu and Birch, 2007).

7. Analysis of yield limiting processes in sugarcane (Appendix 4).

A synopsis of yield contributing and yield limiting processes of sugarcane is required given the difficulty facing many sugarcane industries in improving cane yields and sucrose % cane. Average cane yields in some countries appear to be approaching a ceiling of about 90 t ha^{-1} and sucrose content has been static in many countries for 20 years or more.

Biomass and sucrose yields accumulate with the development of the leaf canopy which progressively intercepts increasing amounts of radiation that is used in photosynthesis to produce sucrose, which is then translocated to various sinks in the plant. The sugarcane canopy develops slowly compared to annual crops such as maize and sorghum. Delayed sugarcane canopy development is due to a slow rate of leaf production and a slow rate of tillering. The daily mean temperature below which leaves stop

elongating and emerging is about 10°C while tillering ceases at a higher temperature of about 16°C. Both tillering and leaf extension respond proportionally to temperature above these lower limits.

Cell enlargement and leaf and stalk expansion are highly dependent on plant water relations. Stalk elongation is more sensitive to water stress than is leaf elongation. Combined leaf and stalk extension can slow when leaf water potential (Ψ_L) declines below –0.2 MPa and can cease when Ψ_L declines below –1.0 MPa. The leaf and stalk extension rate of clones with a high sucrose content were more restricted by the imposition of water stress and reduced temperatures than were clones with low sucrose content. Differences among clones in sensitivity to abiotic environmental stresses may account to some extent for the differences in the concentration of sucrose among clones since decreased cell expansion would require less photoassimilate for cell wall synthesis and thus allow a higher fraction of photoassimilate to accumulate as sucrose in the stalk. Despite its slow rate of canopy development, the long crop cycle of sugarcane makes it highly efficient in intercepting annual radiation compared to other crops. Rates of photosynthesis per unit leaf area for individual leaves were greater for sugarcane than for *Zea* and *Sorghum* spp. Maximum photosynthesis of single leaves is reportedly in the 30 – 35 μmol/m^2.s range while photosynthesis of whole plants is about half this rate.

Radiation use efficiency can be as high as 2 g MJ^{-1} but the average in experimental plots is only about 1.44 g MJ^{-1} because of a developmental slowdown, now called the reduced growth phenomenon (RGP), which appears to start after 30 to 50 t biomass ha^{-1} has accumulated. Factors including lodging, feedback on photosynthesis by sucrose accumulating in the stalk and respiration of sucrose could be responsible for the RGP. The high efficiencies of radiation capture and use in sugarcane are not matched by high efficiencies in the allocation of biomass to sucrose (only 30 to 40%). In commercial cultivars, the fraction of above ground biomass in the stalk is 0.66 to 0.80 and up to 50% of that can be sucrose.

Although breeders and physiologist may be able to make incremental gains in the large number of steps required to build biomass and sucrose yield, the growing community has a better opportunity to increase yields by crop management changes towards achieving the yield potential already built into the plant through many years of breeding and selection. Significant yield gains with existing cultivars should be possible since in at least one study only 53 to 69 % of the potential was achieved. One way to direct crop management changes is to use growth models to estimate target yields that could be achieved with given radiation, temperature, rainfall and irrigation regimes and then to work with growers to identify fields that are under-performing in order correct possible limiting factors.

INTELLECTUAL PROPERTY AND CONFIDENTIALITY

There are no intellectual property issues associated with this project. The results will be made available to all through progressive publication.

ENVIRONMENTAL AND SOCIAL IMPACTS

This project is of long-term strategic significance (horizon 3) and has no negative environmental or social impacts.

EXPECTED OUTCOMES

Reduced translocation and consequent build up of sugars in sugarcane leaves results in a negative feedback on photosynthesis and a reduction photosynthesis rates in the afternoon for example. This feedback was demonstrated as far back as the 50's and 60's when sugarcane was a popular laboratory model leading to the discovery of the C4 photosynthetic pathway. This process has again received attention recently in a number of articles in high level journals, this time with a focus on genomics and suggestions of genes and signals that could be targeted to reduce such feedback. The logic has extended to involve the cane stalk in the feedback process leading to the assumption that sucrose accumulation in the stalk feeds back to the leaf resulting in a reduction in photosynthesis with crop age.

Our data confirmed well documented observations about reduced photosynthesis in the afternoon and reduced photosynthesis with crop age but the data did not support the assumption that sucrose accumulation in the stalk is the cause of reduced photosynthesis with age.

This is an important finding and will hopefully influence research programs designed to find and then down-regulate signals and feedback pathways from stalk to leaf. Importantly the work also shows that increased biomass yield from sugarcane may not require substantial reductions in sucrose content.

Comments from breeders about the work (Appendix 5) shows that this finding is important to them and somewhat encouraging in that selection for biomass yield will not necessarily change their selection procedures unduly. It is possible that selection for biomass will lead to higher fibre and lower CCS for other reasons. High fibre content may confer resistance to lodging which has a negative effect on CCS and biomass yield. Also high fibre content could help to reduce respiration losses due to high levels of sucrose as suggested in output 3.

Respiration of sucrose could be responsible for the reduced growth phenomenon that is now widely accepted to occur in sugarcane (van Heerden *et al.* 2010) and is attributed at least partly to feedback inhibition of photosynthesis.   The research conducted in this project should help to moderate this view and thus help to direct research into the real causes of the reduced growth phenomenon.

FUTURE RESEARCH NEEDS

Interest in sugarcane as a feedstock for a biofuel industry has increased over the life of this project (Appendix 6) and one of the milestone criteria was to consider biomass yields in marginal areas (Output 4). When modelling these biomass yields it was clear that we do not know how long stools can survive after harvest without rain. Stool survival will probably be highly dependant on root vigour and rooting depth which will probably vary depending on the extent to which clones are derived from wild types such as *S. spontaneum*. While this project focussed on specific physiological mechanisms that could influence the selection of clones for a biomass industry, many more traits would be of interest for such an industry particularly if it was based on low inputs levels (water, nutrients). The capability to measure and model stool survival in dry conditions seems to be a large gap in our knowledge not only for marginal climatic conditions but also for current climatic conditions in the industry where replanting after drought is sometimes required. Hopefully some information about stool survival will be gained during the 'More crop per drop project' (BSS334) but if not we may need some specific trials in very dry climates to obtain the data required.

The factors involved in the growth slow down phenomenon have yet to be resolved. Research in this project has directed attention away from feedback inhibition towards respiration of sucrose as a possible cause. Measuring respiration in field crops may be quite difficult to achieve so it is suggested that the equations developed in this project be tested using the large number of growth analysis datasets available in Australia and South Africa. After modelling growth slow-down it may be necessary to confirm assumed mechanisms and rate coefficients by conducting targeted field experiments or experiments on single plants or even on plant components.

RECOMMENDATIONS

Scientists at the South African Sugarcane Research Institute (SASRI) were often in engaged in discussions about issues in this project. Research at SASRI on feedback inhibition had a large influence on the objectives and conclusions of this project. Their involvement arose from their interest in overcoming limits to

CCS and solving the reduced growth phenomenon.   Collaborative work with SASRI along the lines suggested above is recommended for solving these limitations.

Recommendations made for the CSE014 final report are still current and it is pleasing to note that SRDC has 'accepted' these recommendations by continuing to support projects with a strong physiology component such as the project on elevated CO_2 (CPI018) and the 'More crop per drop project' (BSS334) which is considering traits for drought resistance. Our knowledge base on traits for sucrose accumulation and drought resistance is growing substantially as a result of these projects and we have tried and will continue to try to get as much of this knowledge reviewed and published in industry and international journals as possible. Fortunately we have also built some of this knowledge into existing and new models which in theory can be learnt and used by younger scientists coming into the industry. However we have not been successful in attracting younger scientists and students into this type of research as was intended for the CSE023 project and also BSS334. The sugar industry through SRDC recognises this difficulty and has made generous grants available for students directly through the 'Scholarships program' and indirectly through research projects. Given the difficulty of attracting young Australians into the industry SRDC may consider a deliberate strategy to encourage bright young students from other countries to come here and pick up skills from older scientists. These skills will then be available at least somewhere in the world if not in Australia where we would hope these students would stay and contribute in the area of crop physiology and modelling.   Some universities in Brazil have a 'sandwich' post graduate program where students conduct research in another country but start and end their studies in Brazil. This approach would avoid the overseas student fees which have proved to be prohibitive for Brazilian students in the past. We are encouraging young Brazilians to visit and at least complete their graduate degrees in Australia. Is there someway to make them more welcome in the industry?

PUBLICATIONS FROM THE PROJECT

1. **Inman-Bamber NG**, Jackson PA, Hewitt M (2011) Sucrose accumulation in sugarcane stalks does not limit photosynthesis and biomass production. Crop and Pasture Science (in review).

2. **Inman-Bamber NG** (2011). Sugarcane yields and yield limiting processes. In Physiology of Sugarcane. Eds Paul H. Moore , Frikkie C. Botha. John Wiley & Sons, Inc. Io (in press).

3. **Inman**-Bamber NG, Jackson PA, Bonnett G, Morgan T (2011). Have we reached peak CCS? Proceedings Australian Society Sugar Cane Technologists 33 CDROM.

4. **Inman**-Bamber NG, Jackson PA, Bourgault M (2011). Genetic Adjustment to Changing Climates: Sugarcane. In Crop Adaptation to Climate Change Eds Shyam Singh Yadav, Robert Redden, Jerry L. Hatfield, Hermann Lotze-Campen, Anthony Hall. John Wiley NJ ISBN: 978-0-8138-2016-3. 439-447.

5. Singels A, **Inman**-Bamber, NG (2011). Modelling genetic and environmental control of biomass partitioning at plant and phytomer level of sugarcane grown in controlled environments. Crop and Pasture Science Volume **62**, 66 – 81.

6. Singels A, Annandale JG, De Jager JM, Schulze, RE, **Inman**-Bamber NG, Durand W, Van Rensburg LD, Van Heerden PS, Crosby CT, Green GC, Steyn JM (2010). Modelling crop growth and water relations in South Africa: Past achievements and lessons for the future. South African Journal of Plant and Soil **27**, 49-65.

7. **Inman**-Bamber NG, Bonnett GD, Spillman MF, Hewitt ML, Glassop D (2010). Sucrose accumulation in sugarcane is influenced by temperature and genotype through the carbon source-sink balance. Crop and Pasture Science **61**, 111-121.

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LIST OF APPENDICES

Appendix 1. Sucrose accumulation in sugarcane stalks does not limit photosynthesis and biomass production

Appendix 2. Benchmarks for sucrose, fibre and dry matter contents, photosynthesis and transpiration efficiency

Appendix 3. Have we reached peak CCS?

Appendix 4. Sugarcane yields and yield limiting processes

Appendix 5. Project evaluation by Sugarcane Plant Breeders – 2011

Appendix 6. Genetic Adjustment to Changing Climates: Sugarcane.

Appendix 7

Maximum published values for sucrose, dry matter, fibre contents and for photosynthesis and transpiration compared to new values from this project and newly discovered published data

Text in black is copied from Appendix 6 in the milestone 2 report. Text in blue and mauve is new data.

DAS= days after start, FW fresh weight basis, DM = dry matter basis, TVD=Youngest mature leaf

Variety	Sucrose %	Where/how	Reference
N19	54 % DM	N19 internode 11 and 15	Botha and Black (2000)
Co7704	18.87±0.88 %FW	Top 3 internodes at 360 DAS	Venkataramana and Naidu, (1993)
Co7704	20.49±0.41 %FW	Basal 3 internodes at 360 DAS	
Mandalay	1.8%FW	of TVD leaf	Grof et al., (1998)
Q117	1.0%FW	of TVD leaf	
Q117,Q138	15.1%FW	Stalks at 460 DAS	Muchow et al (1996a)
Q117,Q138	48%DW	Stalks at 460 DAS	
N26	55%	12 month whole stalk in August	Singels and Donaldson (2005)
Q117	51.7%DM	13 month whole stalk in August	Muchow et al (1996b)
N varieties	55%DM	Highest of 100s of values	Singels and Bezuidenhout (2002)
Pre-release	17%CCS	Highest of 15 clones	Jackson (2005)
N19	160 umol/g FW	Internode 10 from top	McCormick et al (2008)
Q117	55.0% DM	Internode 12 from top Low temperature	Bonnett et al., (2006).
Q96, Q117	50 % DM	Whole stalks august to Nov where stalk dry mass >30 t/ha	Inman-Bamber et al., (2002)
NCo376	55 % DM	Basal 50cm	
Q96,Q117	18% FW 52% DM	Whole stalk, 11 months, long dry-off	Inman-Bamber (2004)
Q183	47% DM	Basal internodes dried off in glasshouse	Inman-Bamber et al., (2008)
N12	17% FW	Highest 10 cm segments	Inman-Bamber and Wood (1987)
NCo376	16% FW	Highest 10 cm segments	
Best of 50 pre-released clones	23.5% sucrose in juice 60 % DM	juice of wholes stalks	Berding (1997)
Burdekin varieties	17.5 to 18%CCS	For 770 tons cane crushed in Burdekin mills	Inman-Bamber at el (2011)
Q96	19% FW	Whole sound stalk 60 weeks old in September - Kalamia	McDonald (2006)

Q117	21% FW, 67%DW	Basal internodes, constrained elongation in TPF	Inman-Bamber et al., (2009)
GM cane	27% sucrose + isomaltulose	Genetically modified	Wu and Birch (2010)
High sucrose clones	20 to 25 % FW	For 5% of the internodes	This project (see Fig 1 below)
Variety	Hexoses	Where/how	Reference
N19	115 umol/g (2%) FW	Internode 7 from top	McCormick et al (2008)
High sucrose clones	2.5 to 3.5 % FW	For 5% of the internodes	This project (see Fig 2 below)
Variety	Dry matter %	Where/how	Reference
Q117,Q138	32%	Stalks at 460 DAS	Muchow et al., (1996a)
Q117,Q138	32.2%	13 month whole stalk in August	Muchow et al., (1996b)
Q96,Q117	37%	Whole stalk, 11 months, long dry-off	Inman-Bamber (2004)
S. spontaneum crosses	41%	Top 5 % of 30 clones from each of 43 crosses, Macknade	Wang et al (2008)
High fibre clones	36 to 41 % FW	For 5% of the internodes	This project (see Fig 3 below)
Variety	Fibre %	Where/how	Reference
S. spontaneum crosses	29% FW	Top 5 % of 30 clones from each of 43 crosses, Macknade	Wang et al (2008)
High fibre clones	28 to 37 % FW	For 5% of the internodes	This project (see Fig 4 below)
Variety	Photosynthesis matter %	Where/how	Reference
Nif4 japan	36.5 ± 2.2 (μmol CO_2/m2/s)	TVD Leaf segments -0.3 (MPa)	Du et al (1998)
Q183	36 (μmol CO_2/m2/s)	TVD leaf segment	N. G. Inman-Bamber et al (2008)
Q183, Q138	17 (μmol CO_2/m2/s)	Whole plant PAR = 1200 (μmol CO_2/m2/s)	
SP80–3280	33 (μmol CO_2/m2/s)	TVD leaf, 2 x normal CO_2, PAR= 1500 (μmol CO_2/m2/s)	De Souza et al., (2008).
SP80–3280	40 (μmol CO_2/m2/s)	TVD leaf, 2 x normal CO_2, PAR= 2500 (μmol CO_2/m2/s)	
H65-7052	41.0 (μmol CO_2/m2/s)	Leaf and root trimmed, 2 week old seedlings in	Ranjith et al (1995)

Variety	Transpiration efficiency %	Where/how	Reference
H69-8235	43.5 (μmol CO_2/m2/s)	nutrient solution. High N treatment	
CP73-1547	33.0 (μmol CO_2/m2/s)	Leaf 7 from top in 720 ppm CO_2	Vu (2006)
High fibre clones QB01-5 & CT04-495	44 - 46 (μmol CO_2/m2/s)	Leaf 1 9-11 am, before stalk elongation phase	Appendix 1 and data below in Fig 5
Variety	Transpiration efficiency %	Where/how	Reference
CP73-1547	19.6 g CO_2/Kg water	Leaf 7 from top in 720 ppm CO_2	Vu et al (2006)
Q127	8.7 g DM/Kg water at 1 kPa	Paddock scale	Inman-Bamber and McGlinchey (2003)
Nine high sucrose clones	Median 17.4 9th decile 27.2 g CO_2/Kg water	Leaf 1, prior to rapid stalk elongation	See data below in Fig 6
Nine high fibre clones	Median 16.9 9th decile 24.0 g CO_2/Kg water	Leaf 1, prior to rapid stalk elongation	See data below in Fig 6

Raw data from CSE023 experiments

Fig 1. Sucrose content of cane on a fresh mass basis for high fibre (F, red) and high sucrose (S, blue) types of clones. Each point is the result of an HPLC analysis of cross sections from three internodes.
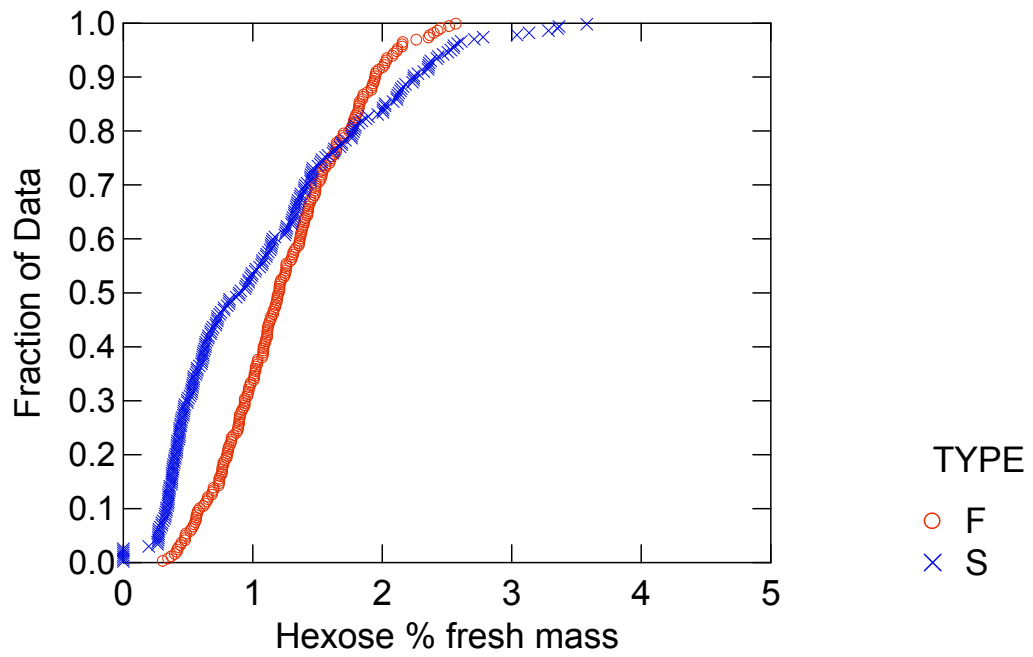
Fig 2. Hexose content of cane on a fresh mass basis for high fibre (F, red) and high sucrose (S, blue) types of clones. Each point is the result of an HPLC analysis of cross sections from three internodes.

Fig 3 Dry matter content of cane on a fresh mass basis for high fibre (F, red) and high sucrose (S, blue) types of clones. Each point is the result of an HPLC analysis of cross sections from three internodes.
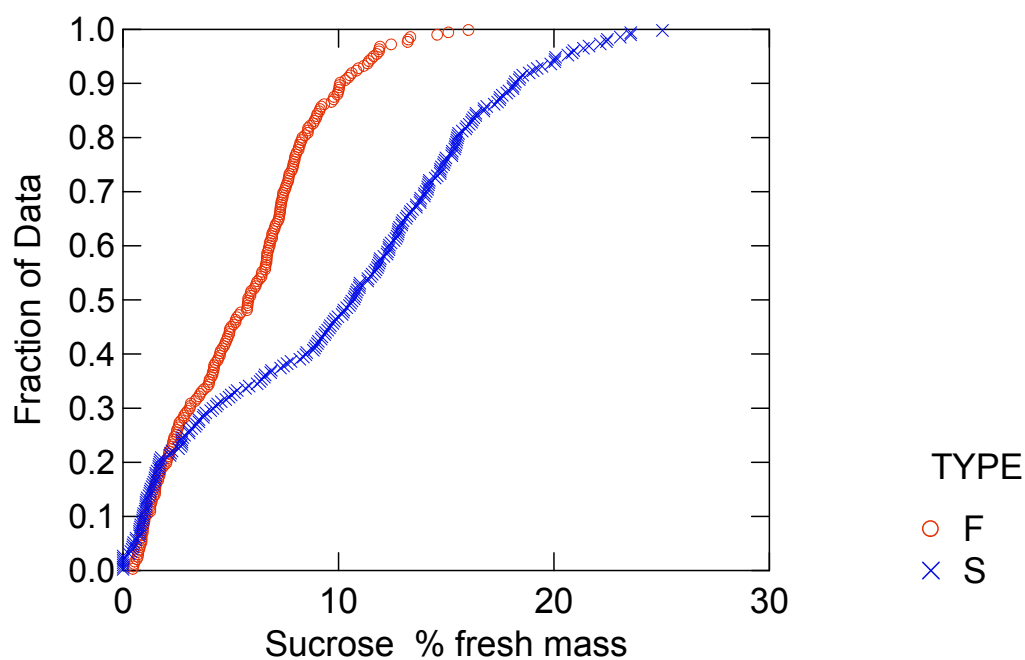

Fig 4. Fibre content of cane on a fresh mass basis for high fibre (F, red) and high sucrose (S, blue) types of clones. Each point is the result of an HPLC analysis of cross sections from three internodes.
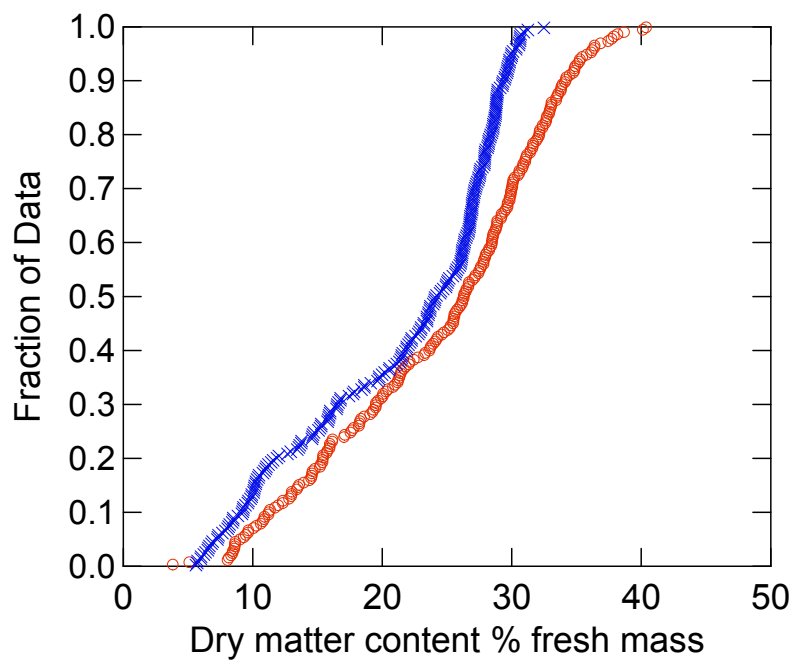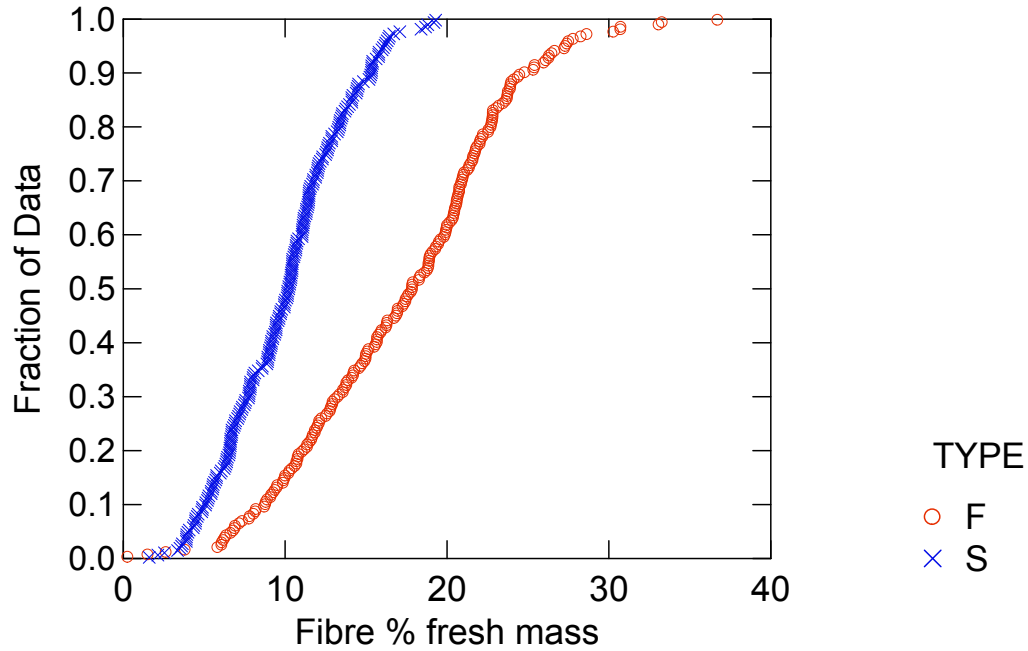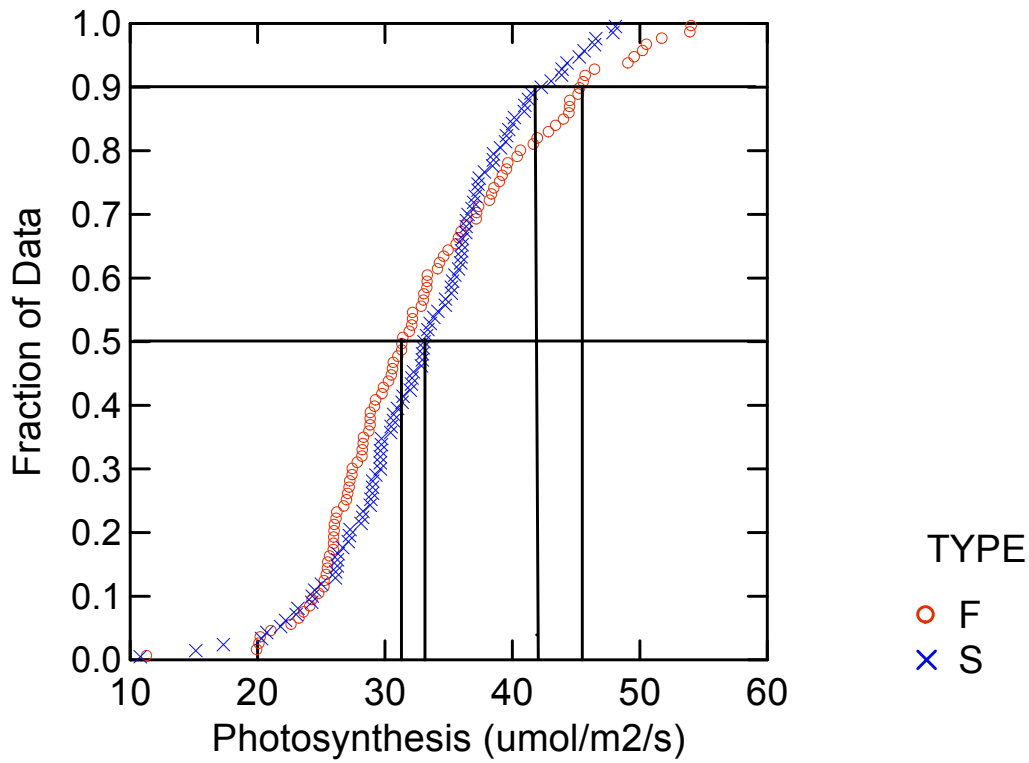
Fig 5. Photosynthesis for leaf #1 for high fibre (F, red) and high sucrose (S, blue) types of clones measured prior to rapid stalk elongation in well watered plants in January – March 2011.
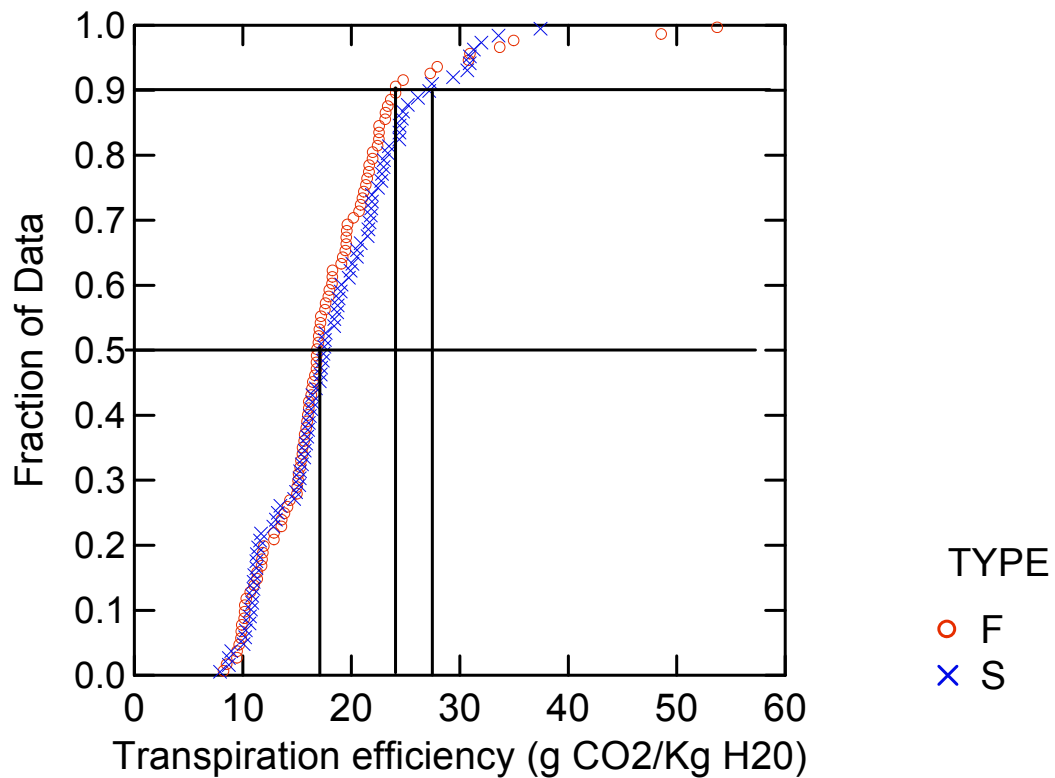
Fig 6. Transpiration efficiency for leaf #1 for high fibre (F, red) and high sucrose (S, blue) types of clones measured prior to rapid stalk elongation in well watered plants in January – March 2011.

Text from Milestone 2 [with new benchmarks inserted in bold type]

The maximum recorded sucrose content obtained in a reliable way (HPLC) was reported by Berding (1997) while examining 154 unselected clones in a selection trial at Mulgrave, Australia. The juice from whole sound stalks contained 23.5% sucrose and sucrose was 60% of stalk dry matter for one clone (2 replications). [This remains as the record for sucrose content of whole sound stalks.] Sucrose was 58% of stalk dry matter for another 11 clones in this trial. The highest sucrose content of fresh cane stalks (20.5 %) was reported by Venkataramana and Naidu (1993) for basal internodes of Co7704. [Sucrose content determined by HPLC reached 21% of fresh weight and 67% of dry weight in basal internodes of Q117 where expansive growth had been constrained by water stress in the TPF (Inman-Bamber et al., (2011).] These authors also measured very high sucrose in the top three (distal) internodes (18.9%) but no one else has published values anywhere near this for internodes at the top of the stalk. It is not clear what was meant by the top three internodes in the paper by Venkataramana and Naidu (1993) and it is possible that they topped the stalk much lower than at the apical meristem (at the base of the youngest mature leaf) which is the accepted 'top' in more recent publications. [Sucrose content in the top 5 internodes was always low and subject more to environment than to genotype even with the wide range of clones used in this project. Wu and Birch (2010) published a record 27% for sucrose content plus its isomer isomaltulose in genetically modified sugarcane plants]

Dry matter content in commercial sugarcane cultivars was as high as 37% after a long and moderate dry off period Inman-Bamber (2004) but Wang et al. (2008) reported dry matter contents up to 41% in crosses between S. officinarum and S. spontaneum. These clones also presented very high fibre contents of up to 29% on a fresh mass basis. [Our maximum dry matter content was 41 % for a basal internode of a high fibre clone]

Maximum photosynthesis rates for sugarcane have been reported under elevated CO_2 conditions and for plants which have been tampered with in some way.. All high rates (up to 43.5 μmol CO2/m2/s) were for small patches (2-10 cm2) of young leaves, normally the youngest mature leaf. [High fibre clones QB01-5 and CT04-495 had mean photosynthesis rates of 44 and 46 μmol CO2/m2/s for leaf #1 measured between 9 and 11 am, before stalk the elongation phase. Afternoon readings were lower]

Photosynthesis on a m2 leaf area basis is expected to be considerably lower for whole plants than for leaf segments and ours is the only recent work reporting such measure reaching 17 μmol CO2/m2/s (Inman-Bamber et al., 2008) at a light intensity of 1200 μmol CO2/m2/s and 18.6 μmol CO2/m2/s at light saturation.

Transpiration efficiency (TE, ratio of photosynthesis to transpiration) has seldom been reported and a benchmark value of 19.6 g CO2/Kg water was found for plants growing in 2 x ambient CO_2 concentration. . [Nine high sucrose clones had a median TE of 17.4 and 9th decile of 27.2 g CO2/Kg water for leaf 1, prior to rapid stalk elongation] The benchmark in terms of aerial biomass accumulation (8.7 g dry mass/Kg water at 1 kPa vapour pressure deficit) was established in our work also funded by SRDC (Inman-Bamber and McGlinchey, 2003). This value is higher than the previous benchmark (8.0 g dry mass/Kg water) assumed by Keating et al. (1999) for use in the APSIM-Sugarcane model

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Appendix 5

Project evaluation by Sugarcane Plant Breeders
2011

At the start of the project a detailed questionnaire (below) was circulated to all breeders in the industry. The same set of questions was sent again in April 2011 (Table 1) with an additional short list of questions to ascertain to what extent the project had revised their baseline knowledge, aspirations, skills and attitudes in regard to photosynthesis and sucrose/fibre accumulation. Three breeders had joined the industry in the meantime but they were also asked these questions as to how they may have responded at the start of the project. The main findings of the project have only recently been collated in the form a journal paper. The key findings were summarised and the abstract of the paper was circulated as well as the full paper to allow the respondents to judge for themselves the impact of the project on their work.

Baseline survey questions

1. Have you heard or read about feedback inhibition of photosynthesis in:
        a. Crops other than sugarcane   not at all ☐ vaguely ☐ much☐
        b. In sugarcane   not at all ☐ vaguely ☐ much☐
        c. Within the leaf of any crop in relation to sugars or starch
                                          not at all ☐ vaguely ☐ much☐
        d. Between sucrose in the stalk and leaf photosynthesis
                                          not at all ☐ vaguely ☐ much☐

2. By breeding and selecting for high CCS in sugarcane, sucrose can accumulate in stalks to such an extent that photosynthesis will slow down because of feed backs along path from sucrose production in the leaf to sucrose storage in the stem.

Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐

3. Photosynthesis slows down with crop development for these reasons unrelated to feedback inhibition.
     a.   A highly conserved reduction in leaf N content with age
Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐
     b.   Unavoidable water stress (increasing distance from soil to leaf for example)
Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐
     c.   Hormonal responses or sucrose signalling involving
Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐
     d.   There is no evidence for reduced inherent photosynthesis with age
Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐

4. Difficulties in improving CCS through breeding and selection are due to feedback inhibition

Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐

5. CCS improvement is no more difficult than improvement of other traits (cane yield, disease resistance)

Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐

6. Dry biomass yields would improve if we selected for high fibre content rather than for CCS
Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐

7. If I were selecting for a biofuels industry based on fibre and sucrose I would look for high fibre clones in the genepool

Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐

8. High fibre types will be more drought resistant

Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐

9. In you experience what is maximum measurement and what do you think is the upper physiological limit for these components in sugarcane.

	Max measured	Upper limit
Brix content (%)		
Sucrose content of fresh mass (%)		
CCS (%)		
Fibre content (%)		
Stalk dry matter content (%)		

10. Storing biomass as sucrose attracts a respiration cost whereas biomass as fibre may not, so dry biomass yields are likely to be higher in high fibre clones than in high sucrose clones regardless of feedback inhibition.

Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐

11. The production of fibre in the plant requires more energy (through respiration) than does the production of sucrose, so biomass yields will be higher if we select for CCS as we do now.

Strongly disagree ☐disagree ☐ not sure ☐ agree☐ strongly agree☐

12. Research on feedback inhibition and respiration in relation sucrose and fibre accumulation will be:
Directly useful for my selection work   ☐
Interesting but not directly useful   ☐
Not relevant at all to me   ☐
Not relevant to anybody in sugarcane improvement   ☐

Table 1. List of breeders surveyed in April 2011

Breeder	Agency	Response
Felicity Atkin	BSES	No
Georg Piperidis	BSES	Yes
Hu Fengduo	BSES	No
John Foreman	CSIRO	No
Mike Cox	BSES	No
Phillip Jackson	CSIRO	No
Prakash Lakshmanan	BSES	Yes
Roy Parfitt	BSES	Yes
Terry Morgan	Sucrogen	No
Xianming Wei	BSES	Yes

Responses

The responses from each scientist are shown against the questions in bold type.

1) George Piperidis

Please look over your response (attached) to the last survey and respond as follows.

1. My views have not changed since the last survey
2. My views have changed [Please say which] I certainly feel much more informed and can see the value of this research.

Please have a look at the abstract below and if necessary the attached draft paper and respond below:

1. The results appear to interesting and could be relevant to my work Yes
2. The results appear to interesting but have no relevance to my work
3. The results are irrelevant to me

Please say whether or not the key finding mentioned above has changed your views about the survey questions.

1. The key finding did not changed my views
2. The key finding made a difference to my views Yes

Just one more response please:

1. I have no further interest in this work or the paper
2. I am interested in this work and intend to read the paper when published. Yes

Kind regards,

George

_____

2) Xianming Wei

Please look over your response (attached) to the last survey and respond as follows.

1. My views have not changed since the last survey
2. My views have changed [Please say which]

I was not sure how the sucrose accumulation evolved with the development of sugar cane, I think I know slightly more.

Please have a look at the abstract below and if necessary the attached draft paper and respond below:

1. The results appear to interesting and could be relevant to my work

It's surely relevant to my work on sugarcane improvement, I think the real issue is how to use the results in a breeding program.

2. The results appear to interesting but have no relevance to my work

3.   The results are irrelevant to me

Please say whether or not the key finding mentioned above has changed your views about the survey questions.
1.   The key finding did not changed my views
2.   The key finding made a difference to my views Yes.

Just one more response please:

1.   I have no further interest in this work or the paper
2.   I am interested in this work and intend to read the paper when published.
Will read the paper.

Any other comments will be welcome.

_____

3) Prakash Lakshmanan

Please look over your response (attached) to the last survey and respond as follows.

1.   My views have not changed since the last survey
2.   My views have changed [Please say which] . Did change my view of biomass crop characteristics, but not surprising that P and sucrose accumulation are getting delinked in the last phase of crop cycle as plants age as Rubisco get shunted out for N as part of the normal housekeeping and to support the limited growth activity. In terms of energy content, lignified tissue is better than sugar, and hence it make sense for selecting high fibre cane for bioenergy/biomass. Interesting to see that there is no feedback inhibition of P.

Please have a look at the abstract below and if necessary the attached draft paper and respond below:

1.   The results appear to be interesting and could be relevant to my work . Yes, **particularly for water stress project.**
2.   The results appear to interesting but have no relevance to my work
3.   The results are irrelevant to me

Please say whether or not the key finding mentioned above has changed your views about the survey questions.

1.   The key finding did not changed my views
2.   The key finding made a difference to my views: yes

Just one more response please:

1.   I have no further interest in this work or the paper

2.   I am interested in this work and intend to read the paper when published. YES, very much, and as much you are!!

Prakash

---

4) Roy Parfitt

Please have a look at the abstract below and if necessary the attached draft paper and respond below:

1.   The results appear to interesting and could be relevant to my work
2.   The results appear to interesting but have no relevance to my work
3.   The results are irrelevant to me

The results definitely appear to be interesting. Presently, my main focus is breeding and selection for smut resistance (SmutBuster program), but as this project starts to wind down and I get more involved in the core breeding program your research will become more relevant.

Please look over the survey questions and say whether or not the key finding mentioned above would have changed your views about the survey questions.

1.   The key finding did not changed my views
2.   The key finding made a difference to my views

Do not think the key findings would have significantly changed my views about the survey questions.

Just one more response please:

1.   I have no further interest in this work or the paper
2.   I am interested in this work and intend to read the paper when published.

I am interested in this work intend to read the paper when published.

Any other comments will be welcome.

---

5) Phil Jackson
Phil responded directly on the original survey sheet (Fig 1).

Questions

1. Have you heard or read about feedback inhibition of photosynthesis in:
   a. Crops other than sugarcane   not at all ☐ vaguely ☑ much☐
   b. In sugarcane   not at all ☐ vaguely ☑ much☐
   c. Within the leaf of any crop in relation to sugars or starch
                                       not at all ☐ vaguely ☑ much☐
   d. Between sucrose in the stalk and leaf photosynthesis
                                       not at all ☐ vaguely ☑ much☐

2. By breeding and selecting for high CCS in sugarcane, sucrose can accumulate in stalks such an extent that photosynthesis will slow down because of feed backs along path from sucrose production in the leaf to sucrose storage in the stem.

   Strongly disagree ☐ disagree ☐ not sure ☑ agree☐ strongly agree☐

3. Photosynthesis slows down with crop development for these reasons unrelated to feedback inhibition.
   a. A highly conserved reduction in leaf N content with age
   Strongly disagree ☐ disagree ☐ not sure ☑ agree☐ strongly agree☐
   b. Unavoidable water stress (increasing distance from soil to leaf for example)
   Strongly disagree ☐ disagree ☑ not sure ☐ agree☐ strongly agree☐
   c. Hormonal responses or sucrose signalling involving
   Strongly disagree ☐ disagree ☐ not sure ☑ agree☐ strongly agree☐
   d. There is no evidence for reduced inherent photosynthesis with age
   Strongly disagree ☐ disagree ☐ not sure ☑ agree☐ strongly agree☐

4. Difficulties in improving CCS through breeding and selection are due to feedback inhibition
   Strongly disagree ☐ disagree ☐ not sure ☑ agree☐ strongly agree☐

5. CCS improvement is no more difficult than improvement of other traits (cane yield, disease resistance)
   Strongly disagree ☐ disagree ☑ not sure ☐ agree☐ strongly agree☐

6. Dry biomass yields would improve if we selected for high fibre content rather than for CCS
   Strongly disagree ☐ disagree ☐ not sure ☑ agree☑ strongly agree☐

7. If I were selecting for a biofuels industry based on fibre and sucrose I would look for high fibre clones in the genepool
   Strongly disagree ☐ disagree ☐ not sure ☐ agree☑ strongly agree☐

8. High fibre types will be more drought resistant
   Strongly disagree ☐ disagree ☐ not sure ☑ agree☐ strongly agree☐

Fig 1. Phil Jacksons response to survey questions in 2009 (tick marks) and responses in 2011 (green highlight)