

SRDC Research Project Final Report

Increased CCS, cane yield and water use efficiency by exploiting interactions between genetics and management

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EXECUTIVE SUMMARY

Issue

In October 2003 delegates at an international workshop on sugarcane physiology funded by SRDC concluded that priority should be directed at a better understanding of traits responsible for high yield and high sucrose content, in order to better design future genotypes. While pathways of sucrose accumulation were and are being investigated at the molecular and cellular levels, there was no concurrent work at the crop level. Consequently it is difficult to answer the questions- To what extent will genetic improvements be modified by management and the environment? and conversely, To what extent does management and environment influence the selection of varieties? These and other questions about the genotype x environment (GxE) interaction on sucrose accumulation and lodging were the major concerns of this project with sucrose accumulation as the predominant issue.

This project forms part of a large effort now underway worldwide to find alternative methods to develop cultivars with improved sugar content. The main objective was to better understand the interactions between sugarcane genetics and the environment (including management) with respect to sugar accumulation. After four years of intensive work the project has delivered extensive new knowledge on the physiology of interactions between sugarcane genetics and the environment with respect to sugar accumulation and lodging and as such has met the main objective of the research.

Methodology

Four major approaches were adopted for the fulfilment of project objectives. Seven controlled glasshouse experiments were conducted to study the GxE interaction on sucrose accumulation (1) a process level model was built to account for allocation of products of photosynthesis to internodes (2), four field plot experiments were conducted to assess the GxE impact on lodging (3) and a series of workshops, presentations, papers and surveys were conducted to communicate and evaluate findings from the project (4). A cycle of planning with the steering panel, execution of the plan and reporting the findings to the steering panel was followed for each year.

Outputs

Outputs from the project are recorded in five journal papers in various stages of publication, two have been published already. Six refereed conference papers were also presented and five were published. The report lists 37 outputs and a summary of the most important ones is given here.

The first glasshouse experiment proved that reduced vegetative growth (stalks and leaves) without a matching reduction in photosynthesis would increase sucrose yield and sucrose content. The additional sucrose came from products of photosynthesis (carbohydrate) that would otherwise have been used to produce additional leaf and stalk tissue. In farming practice irrigation could be applied so as to regulate vegetative growth down by about 30%, during dry periods throughout the crop cycle, without an equivalent reduction in photosynthesis or biomass accumulation, leading to higher sucrose content and higher sucrose yield.

Differences in sucrose accumulation between high- and low sucrose clones were not due to differences in the rate of photosynthesis. The mass of tissue yet to be 'filled' with photo-assimilate was greater for the low than the high sucrose clones but this did not favour increased photosynthesis of whole plants which is counter to expectations from some recent publications suggesting that photosynthesis is reduced as stalk fill up with sucrose.

Clones with low sucrose content can accumulate sucrose equivalent to a highly selected, high sucrose clones, albeit in only a limited number of basal internodes. This suggests that the maximum amount of sucrose that can accumulate in stalk tissue is not the limiting step for sucrose accumulation. The difference between high- and low sucrose clones could be explained purely by the partitioning of carbohydrates between storage and growth. The biochemistry of tissue which is up- or down regulated for parts of the sucrose accumulation pathway may then be an effect rather than a cause of the difference.

Differences between clones in the glasshouse were similar to their differences observed in the field. Furthermore the magnitude of many yield components was similar for the glasshouse and the field if one pot was assumed to represent one square metre of a cane paddock. Thus results from these glasshouse studies are relevant to field situations at least for the traits being investigated. The precision of the glasshouse measurements made it possible to distinguish reliably between clones for many of the traits observed including those involved in sucrose accumulation. The ability to distinguish easily between clones allowed us to make reliable distinctions between groups or types of clones. High sucrose types had more green leaves per stalk but they allocated proportionally less biomass to leaves and leaf sheaths (cabbage) as well as to dead leaves so additional biomass could be allocated to the stalk component.

A mathematical model was developed to explain observations of sucrose accumulation in internodes of clones, varying widely in sucrose content. The internode model worked well and supported the hypothesis that sucrose accumulates after other priorities for carbohydrate have been met. Thus genes controlling the level of demand for these priorities are at least as important as those for sucrose accumulation in storage tissue.

The research indicated that erect and retarded crops can ‘catch up’ with more advanced and lodged crops after the wet season but this did not favour clones that were more prone to lodging. The research proved that at least in some soils, growers could reduce irrigation amounts considerably without significant yield loss and it also proved that lodging can be delayed by withholding irrigation to some extent before the wet season. WaterSense can be used to help growers to irrigate for maximum CCS and to reduce lodging.

Impacts

When this project commenced, sound physiological explanations for differences in sucrose accumulation and sucrose content among sugarcane clones and cultivars did not exist despite the considerable effort to identify genes, enzymes, and biochemical pathways that might differ among high- and low-sucrose genotypes. The project raised important questions about where research at cellular and sub-cellular levels should be directed and the relative importance of this level of investigation compared to studies at the whole plant level.

This project and its predecessor (CSE006) gave rise to a large number (20) of journal articles. Papers published in a special issue of Field Crops Research in 2005, have been cited 131 times to date with an impact factor of 7.2, more than three times higher than for the journal itself. Sugarcane physiology research, while limited in Australia compared other countries, has attracted considerable attention, thus allowing us to freely exchange knowledge generated in these other industries.

Benefits of this strategic project (horizon 3) are expected in the long term and the pathway to these benefits has been defined in terms of the modelling framework required to link knowledge across different levels of organisation of the plant. Australia has taken the lead in this type of gene to phenotype modelling in other crops where the vision is to design crops to match variable economic and environmental requirements by

understanding the role of genes or markers in relation to individual traits. We have made a good start with this project, in catching up with other cropping systems in linking genetics and the environment (including management) through crop and plant physiology, having begun to fill the gap in knowledge clearly identified at the 2003 physiology workshop.

Despite the strategic nature of the project some results can be applied immediately as demonstrated in this report on how WaterSense can now be used to control elongation growth, lodging and CCS in farming practice.

BACKGROUND

Sugarcane received attention for a short while as a model species for studies in plant physiology during breakthroughs on photorespiration and the discovery of the C₄ photosynthetic cycle (Kortschack *et al.* 1965; Hatch and Slack 1966). Since then progress in sugarcane physiology at all levels (molecular to crop) has fallen behind that of other major crops. The proliferation of crop simulation models in the 1980s and 1990s led to some basic investigations in order to provide rate coefficients for sugarcane growth models. At about the same time sugar industries in several countries started investing in biotechnology for sugarcane and the investment and intensity of this research has increased remarkably with a consequent reduction in emphasis on ecophysiology. Modelling and molecular technologies took sugarcane physiologists in these two different directions and little research was done outside these two fields. While crop simulation technology was able to help solve some sugarcane production problems, the solution to other issues was frustrated by the lack of basic physiological information. In Australia low yields and low sucrose content in extreme climates (high temperature or high rainfall) could not be explained by models or current knowledge of plant or crop physiology (Wilson and Leslie 1997; Leslie and Byth 2000).

The size and complexity of the polyploid genome of sugarcane means that the genetic tools available to sugarcane scientists also lag behind those of model plants and other crops such as Arabidopsis and rice. In these plants, the sequence of the whole genome and an increasing number of characterised mutants are available for studying the connection between particular genes, physiology and phenotype. Such tools are not yet available for sugarcane.

In recognition of the comparatively poor state of physiological knowledge in sugarcane and the divergence in the research areas at the molecular to whole crop scales, the Sugar Research and Development Corporation (SRDC) of Australia provided funds for an international workshop on sugarcane physiology, with the theme of integrating knowledge from cell to crop to advance sugarcane production (project CSE006). The goal of the workshop, which was attended by about 40 delegates, was to identify the critical gaps in our understanding of plant and crop physiology for sugarcane that, if addressed by research, could lead to advances in the economic and environmental sustainability of sugarcane production. The workshop identified sucrose storage as the most important process for future research. Water and N use efficiency were also given high priority. Genetic and environmental controls of these processes were highlighted as priorities for future research. Physiological and morphological traits responsible for improved yield, sucrose content and resource use are poorly understood in sugarcane. Delegates reached a consensus that effort should be directed at a better understanding of traits responsible for high yield and high sucrose content and high water and N use efficiency, in order to better design future genotypes.

While pathways of sucrose accumulation were and are being investigated at the molecular and cellular levels, there was no concurrent work at the crop level before the project now being reported was initiated. Genes can be regulated by external influences acting on the plant such as temperature and water stress. To what extent will genetic improvements be modified by management and the environment? Conversely, to what extent does management and environment influence the selection of varieties? How come released varieties lodge so readily when lodging is a severe constraint to yield

(Singh *et al.* 2002). These questions about the GxE interaction on sucrose accumulation and lodging were the major concerns of this project. While sucrose accumulation was the predominant issue, the GxE interaction on lodging was also given considerable attention because of interest from leading breeders and agronomists in Australia.

Sucrose accumulation

In the Australian sugar industry as in many others, the value of the crop delivered to the mill is measured in terms of commercial cane sugar (CCS) in which sucrose content is the dominant factor. An increase in sucrose yield due to improved CCS is up to 1.8 times more valuable than a sucrose yield increase due to improved cane yield (Jackson *et al.* 2000). This is because increased cane yield attracts marginal costs in harvesting, transport and milling whereas an increased CCS does not. Unfortunately in Australia, recent improvements in sugar yield have been achieved through cultivars with improved cane yield rather than improved CCS (Jackson 2005) and this is a common feature in sugarcane industries around the world (Moore 2005).

A large effort is now underway to find alternative methods to develop cultivars with improved sugar content. One method is to increase sucrose content by incorporating more accessions of the progenitor basic germplasm, facilitated by molecular markers (Aitken *et al.* 2006). Another is utilising cell biology and genomic approaches to identify genes that are involved in, or correlated with sucrose accumulation (Casu *et al.* 2004, 2005; Rae *et al.* 2005; Watt *et al.* 2005).

However, there is most certainly a physiological limit to the amount of sucrose that can be stored in a given volume of cane stalk. The highest reported sucrose content of dry matter (SCd) for whole stalks of field grown sugarcane were found in unselected clones by Berding (1997). SCd was as high as 600 mg g⁻¹ in one clone but was generally in the 500 to 560 mg g⁻¹ range, which is still higher than normally achieved commercially. Sucrose content depends greatly on the stage of development and general growing conditions as well as on the genotype. Analysis of sections of cane stalks demonstrated the 'dilution' mechanism responsible for the overriding effect of cane mass (or height) on SCd (Inman-Bamber *et al.* 2002). Increasing whole stalk SCd in sugarcane occurred in two phases, one in which SCd of basal internodes was increasing and the other in which SCd of basal internodes had reached a maximum. In the second phase, further increments in SCd of whole stalks depended mostly on the rate of SCd increase in distal internodes. Once the crop was through the first phase, seasonal variation in SCd of whole stalks was largely due to partitioning to sucrose in distal internodes mediated by factors such as water and nutrient stress and temperature which affect expansive growth more than photosynthesis (Inman-Bamber *et al.* 2002). Singels and Bezuidenhout (2002) developed equations for this concept and distinguished between theoretical cultivars on the basis of their response to ripening stimuli such as low temperature or water stress. They postulated that cultivars with high whole-stalk SCd have responded to ripening stimuli more than those with a low whole-stalk SCd and display a steeper downward gradient in SCd toward the top of the stalk (Singels *et al.* 2005).

Various studies indicate that partitioning to sucrose in the cane stalk is related to the demand for assimilate by other organs. Glaziou and Gayler (1972) transferred sugarcane plants growing at 17°C constant temperature to 30°C and sucrose content on a fresh weight basis (SCf) fell from 16.0 to 6.5 % over a period of 35 days indicating that rapid growth at high temperature depended on carbohydrate stored in the stalk. Conversely when leaf growth is suppressed, sugars tended to accumulate in the stem. In a rainout shelter experiment where some plots were irrigated frequently and others were denied water for five months, stomatal conductance responded to treatment about two weeks after responses in plant extension rate (PER) were noted and conductance was reduced only 50% when PER reached zero (Inman-Bamber 1995; Inman-Bamber and Smith 2005). Two months after expansive growth had ceased, conductance was still about 1.5 cm/s compared with 3 cm/s for irrigated plants and SCf was 14.3% for stressed plants compared to 10.3% for well watered plants (Inman-Bamber and Smith 2005). In regard to sucrose yield, the increased SCf in these South African cultivars was almost sufficient to offset the large reduction in cane yield caused by water stress (108 vs 75 t/ha). In a similar

experiment on Australian cultivars, SCd was increased from about 430 to 500 mg/g and sucrose yield was increased as much as 3.6 t/ha by withholding irrigation. PER in the stressed plants had been reduced 80% for about 6 weeks when the treatments differed most (Inman-Bamber 2004).

Partial control of expansive growth is commonly practiced when growers withhold irrigation or apply ripeners to limit leaf and stalk elongation shortly before harvesting (Robertson and Donaldson 1998). It seems that these practices benefit SCd and SCf by reducing the rate of increase in stalk volume and leaf area (expansive growth) sooner than photosynthesis hence increasing the accumulation of stored sucrose that would otherwise have been translocated to meristematic regions. If expansive growth, of which PER is one measure, could be limited throughout the growth cycle without limiting photosynthesis it may be possible to grow smaller crops with high SCd and SCf without limiting sucrose yield. As far as is known this has never been attempted.

In Australia it is common to recommend irrigation when stalk elongation falls to 50 % of the maximum elongation observed since the last irrigation (Holden 1998) the assumption being that a 50 % reduction in stalk growth will not reduce sucrose yield (G. Kingston pers. comm. 2002). This assumption was shown to be conservative in a dry-down experiment which indicated that biomass accumulation would not be reduced until stalk elongation rate dropped to 30% of the rate obtained with adequate water (Inman-Bamber 2004). If PER decreases at a constant rate after irrigation as suggested by data from Inman-Bamber (2004) and Inman-Bamber and Smith (2005) then we could argue that PER could be reduced by 25 to 35% on a daily basis without reducing sucrose yield. A shorter crop with a higher sucrose content should result. Depending on the extent to which photosynthesis is affected, mild water stress could actually increase sucrose yield because assimilate that would have been used for cell wall synthesis (fibre) can be stored in existing stalk tissue.

Lodging

Lodging reduces benefits from sugarcane production in three ways. CCS can be reduced when lodged cane is harvested because of dilution by the presence of extraneous matter in the form of, leaves, cabbage, and sucker culms (Berding *et al.* 2002). Increased non-stalk material in the harvested crop biomass dilutes sucrose content of mature stalks, inflates fibre content, and increases colour precursors in crystal sugar (Berding 2005). Two additional mechanisms operate to reduce yield prior to harvesting after partial or complete lodging. Cane quality and yield are reduced, through increased stalk death and reduced photosynthesis (Berding 2005). When cane was held erect with bamboo scaffolding in a series of experiments, sucrose content (or CCS) increased by 3-12%, and sucrose yield by 15-35% (Singh *et al.* 2002). Berding and Hurney (2005) devoted considerable attention to component traits (stalk height, number, diameter and shear strength) that could be responsible for the unwanted lodging trait. Stalk height was the only component trait that was related to lodging. Early selection of erect clones on the basis of stalk height would guarantee clones with short stalks and probably low yield. Berding and Hurney (2005) suggested that a combination of breeding and management approaches need to be used to reduce yield losses due to lodging. One of the ways in which lodging can affect yield is through the disruption of the canopy and therefore reduced radiation use efficiency.

OBJECTIVES

To increase sugar yield per ha through increased CCS and cane yield by capitalising on better understanding of the interactions between genetics and management. Specifically:

- To better understand the interactions between sugarcane genetics (gene expression) and environment (including management) with respect to sugar accumulation (which is a current gap in knowledge of sugarcane physiology);
- To improve variety choice and cane management for increased CCS and cane yield
- To determine improved selection systems to identify elite varieties with desired characteristics
- To develop novel irrigation strategies to prevent lodging and maximise CCS
- To promote better management practice for maximising sugar yield and reducing water use

The report that follows shows that this project has delivered extensive new knowledge on the physiology of interactions between sugarcane genetics and the environment with respect to sugar accumulation and lodging and as such has met the main objective of the research. The report will also demonstrate delivery of novel irrigation strategies to prevent lodging and maximise CCS and how these have been promoted to encourage better management for maximising sugar yield and reducing water use. Physiological knowledge was advanced sufficiently to account for genetic and environmental influences on sucrose accumulation in internodes of sugarcane but we were not able to incorporate the model developed for this purpose into the APSIM-Sugarcane model in the time frame of the project. The report will show how international efforts for understanding the physiology of the GxE interaction aim to develop technology for 'designing' sugarcane varieties for specific environments as well as for enabling growers to choose the best combination of varieties on the farm. The project was constructed and executed with this ambitious aim in mind but a lot more work is required internationally to achieve this goal not only for sugarcane but for all important food and fibre crops.

METHODOLOGY

Four major approaches were adopted for the fulfilment of project objectives. Controlled glasshouse experiments were conducted to study the GxE interaction on sucrose accumulation (1) a process level model was built to account for allocation of photosynthate to internodes (2), field plot experiments were conducted to assess the GxE impact on lodging (3) and a series of workshops, presentations, papers and surveys were conducted to communicate and evaluate findings from the project (4). A cycle of planning with the steering panel, execution of the plan and reporting the findings to the steering panel was followed for each year of the four-year project except for reporting to the steering panel in the last year which will occur after the due date for the final report. While the four approaches were highly interrelated, methods for each will be presented separately for clarity.

Glasshouse work on sucrose accumulation

The plan for research on sucrose accumulation which constituted the main thrust of the project was to publish findings for each year in a highly respected international journal. Papers for years 1 and 2 are already in print and a paper for year 3 has been prepared for review and research conducted in year 4 has been formulated in terms of a draft for a journal article. Methods will be reported under headings for each year as a summary of the full length paper published or in preparation for that year. Journal papers in various stages of publication are presented in full as appendices.

Year 1 (2005//06) – Appendix 1

The research conducted in the first year was aimed at developing a methodology to assess variation in response to source-sink manipulation in sugarcane clones. Depending on the extent to which

photosynthesis is affected, mild water stress could actually increase sucrose yield because photo-assimilate that would have been used for cell wall synthesis (fibre) can be stored in existing stalk tissue. An experiment was designed to limit expansive growth more than photosynthesis to test the above hypothesis. Plant extension rate (PER) was selected as the measure of expansive growth because it is highly responsive to temperature and water supply (Inman-Bamber and Spillman 2002). As temperature and water supply are difficult to control in the field, the research was conducted in the Tall Plant Facility (TPF) at CSIRO's Davies Laboratory in Townsville. Temperature has been demonstrated to be well controlled in this facility (Bonnett *et al.* 2006) and because it is enclosed water supply can also be well controlled and photosynthesis can be measured directly through gaseous carbon exchange. The TPF has two chambers which were used for two irrigation or temperature treatments in this project. For this experiment chamber 1 was assigned to the well watered treatment ('wet' treatment) and chamber 2 to the 'dry' treatment which was designed to enhance sucrose accumulation as much as possible.

Plants

Two cultivars were selected for the experiment, one representing high CCS (Q183) and the other low CCS (Q138). On 20 February 2006 when stalks were about 2 m long, 20 pots of each cultivar were transferred to each of two 6.50 m high chambers in the TPF. Pots were mounted on mobile stands about 10 mm from ground level. Stalks were loosely tied to the upright supports of the stands to prevent sprawling or lodging.

Irrigation treatments

All pots were watered automatically 3 times daily until 6 March when pots in the *dry* chamber were watered manually and less often to reduce PER. All pots in the *wet* chamber were irrigated automatically when mean potting mix water content dropped to 34% by volume as determined by reflectometry. Irrigation was applied for 3 minutes which was sufficient to fully wet up the potting mix and allow for some drainage. The trigger for irrigation in the *dry* chamber was adjusted while noting hourly PER readings in order to reduce PER in the *dry* chamber by 25 to 35 % compared to PER in the *wet* chamber. The water content required to trigger irrigation in the *dry* chamber varied between 15% and 8% as experience was gained in controlling daily mean PER.

Temperature and radiation

Long-term mean hourly temperature for March at Kalamia Mill near Ayr, QLD was used to set the temperature regime for each chamber on 21/2/06 (Table 1). These temperatures were reduced by 2 °C on 17 March 2006 because plants were becoming too tall in the *wet* chamber. The TPF control system used these settings to achieve a smooth diurnal temperature cycle similar to ambient conditions outside except that the cycle was the same each day for both chambers.

Table 1. Hourly temperature settings for the TPF chambers

Hour	°C	Hour	°C	Hour	°C
100	24.1	900	27.2	1700	29.2
200	23.7	1000	28.8	1800	28.1
300	23.3	1100	29.6	1900	26.7
400	23.0	1200	30.1	2000	25.9
500	22.9	1300	30.3	2100	25.5
600	22.8	1400	30.4	2200	25.2
700	22.8	1500	30.3	2300	24.9
800	24.5	1600	29.9	2400	24.4

PER measurements

Auxanometers as described by Inman-Bamber and Spillman (2002) were assembled using 10-turn potentiometers with variable electrical resistance (0 to 1 k Ω) depending on the rotation of the spindle. PER readings started on 1 March and continued for the duration of the experiment. An hourly reading was missed twice each week when the auxanometers were attached to new leaves.

Whole plant photosynthesis

External air was passed through each chamber at a known rate via intake fans, plenums and outlet vents. Air speed was measured with small anemometers and with a hot-wire anemometer. A pump and solenoid system was controlled with a data logger to feed a sample of inlet and outlet air simultaneously through the sample and reference tubes (differential mode) of an infra red gas analyser (IRGA). The difference in [CO₂] between intake and exhaust air from one chamber was recorded for 12 minutes and from the other chamber for the next 12 minute period. Net photosynthesis of all plants in each chamber was the product of volumetric airflow and the difference in [CO₂] between inlet and outlet air.

Single leaf photosynthesis

Measurements of photosynthesis were made on individual leaves on 16 and 17 March 06 with a portable photosynthesis meter. The distal end of the youngest fully expanded leaf (leaf 3) was selected for measurement. Five leaves of each cultivar were measured at eight times over two days in the *dry* chamber and six times over two days in the *wet* chamber. Three of the five leaves were tagged for repeated measurements on the same leaf. Readings started at about 9 am and ended at about 1 pm each day.

Destructive sampling

Three pots of each cultivar were removed from each compartment on 2- 3 March 06. Plants were cut at ground level and all the material weighed. Nodes and green leaves were counted on each of the three primary stalks. The length of each stalk was measured to the youngest visible ligule or dewlap (TVD). All leaves were removed from the stalks. Dead leaf and sheath material, green leaf blades, and green leaf sheaths were weighed separately. The area of leaf blades from one stalk in each pot was measured with a planimeter and the position recorded. The mass of these leaves was obtained after drying to constant weight at 80°C. 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 and internodes 2, 4, 6, and 8 were excised and the lengths and diameters measured. Lower internodes were also excised in later samplings. Transverse segments (disks) about 5 mm thick were excised from the middle of each of these internodes. The disks were quartered, placed in vials and immediately frozen in liquid N₂. The remainder of the stalk material was split, weighed and then dried to constant weight at 80°C. This procedure was repeated on five pots of each cultivar from each chamber on 30-31 March and on 20-21 April.

The frozen internode tissue was kept at -80°C until the sucrose and dry matter contents of the sampled internodes were determined. Soluble sugars were extracted by incubating 1.0 to 1.5 g of internode tissue with 9.9 mL water at 70°C overnight after an initial 5 minutes at 95°C. The supernatant was decanted and kept and the tissue pieces extracted a second time in 9.9 mL water at 70°C overnight. The two supernatants were combined and stored at -20°C until analysed. An additional 1.5 to 2.5 g of internode material was weighed, dried to constant weight at 65°C then re-weighed. The dry matter content was then calculated.

Sucrose content was measured in a sample of the extract that had been diluted 2800 times with a mechanical diluter then passed through a 0.2 μ m filter. 10 to 90 μ L of each sample were injected into a HPLC system and sugars were separated on a column protected by a guard column. A mobile phase of

sodium hydroxide was used to separate the sugars which were detected by pulsed amperometric detection and quantified as described by Albertson and Grof (2007).

Sucrose content of dry matter (SCd) of the disks was derived from their sucrose and dry matter contents and was assumed to represent SCd of the entire internode. Mean SCd for the whole stalk, for each sampling date, cultivar, irrigation regime and replication, was derived by interpolation.

Year 2 (2006/07) – Appendix 2

For the second year we proposed that the variation in sucrose content and sucrose accumulation among sugarcane genotypes should be explained by variations in net photosynthesis and in partitioning of photo-assimilate regardless of the nature and importance of mechanisms linking photosynthesis and sucrose storage and regardless of whether sucrose is a priority sink or not. The hypothesis being tested this year was that differences in sucrose accumulation in four clones differing widely in sucrose content could be explained by a source-sink approach.

Selection of four clones

Five commercial cultivars and 29 clones from three biparental crosses (IJ76-514 x Q165^A, KQ99-1401 x Mida^A and MQ77-340 x Q117) were selected for variation in sucrose content and propagated in two replicate blocks at Kalamia Estate, near Ayr on 6-7 September 2005. Plots were 10 m long consisting of 3 rows separated by 1.8 m. Normal estate irrigation and fertilizer were applied. Stalk samples to determine sucrose content were taken from each plot on 22 March 2006 and from only 12 clones with extreme sucrose content on 15 June 2006. In each case 10 stalks were removed, stripped of their leaves and cut 75 cm from the base to obtain sucrose content from top and bottom stalk sections separately. Four clones were selected for the experiment, two (KQ97-2599 and KQ97-2835) at the low extreme of sucrose content and two (Q117 and KQ97-5080) at the high extreme. The propagation trial was harvested on 26 July 2006 and ratooned as a commercial block on Kalmia estate. On 4 June 2007 each plot of the ratoon crop was sampled as before by removing 10 contiguous stalks and then stalks were counted over 5 m lengths of the centre row.

Glasshouse Methods

Methods for pot culture and the experimental procedure and measurements were similar to those reported for year 1. The TPF was programmed to maintain a realistic diurnal temperature cycle that would provide conditions that were intermediate for sucrose accumulation and that would limit maximum extension rate so that roof height (6 m) would not be exceeded. A cooler regime was set on 4 February 2007 when it was apparent that well watered plants would reach roof height before the planned end of the third experiment (Fig. 1).

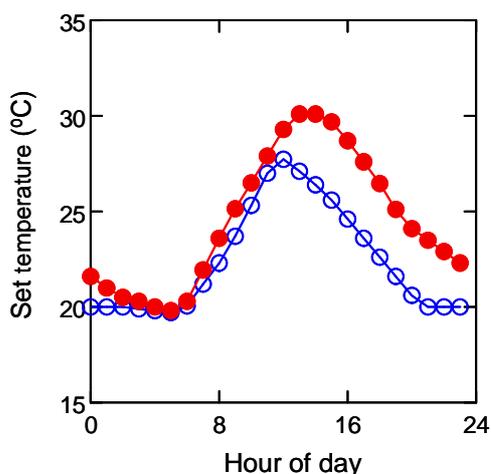


Fig. 1. Hourly set temperature for chambers in the Tall Plant Facility (TPF) for 31 Oct. 2006 to 3 Feb. 2007 (●) and for 4 Feb. 2007 to 4 Jul. 2007 (○).

Plants

For each clone, 200 setts with one node (and bud) were planted in a sand/peat mixture on 11-13 July 2006. After about three leaves had emerged, three seedlings were transplanted into each 27 L pot filled with 25 kg of a commercial potting mix. The pots were placed in the open and were irrigated three 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.

Rotation experiment 1 – photosynthesis measurements

The aim of this experiment was to compare clones in regard to rates of photosynthesis when plants were at about the eight leaf stage prior to the rapid stalk elongation phase. Ten pots of each clone were placed on stands fitted with rollers close to the TPF but still outside in mid October 2006. During November the clones were rotated between two chambers of the TPF and the outside according to a schedule based on a repeated balanced incomplete block design which ensured that net whole plant photosynthesis of each clone was compared with photosynthesis of each other clone on two occasions, once with clone *i* in chamber 1 and clone *j* in chamber 2 and once the other way around.

Intact leaves were counted on each of the three primary shoots and on each tiller in each of 10 pots per clone, at the start of the experiment (31 October to 6 November 2006) and for each of 5 pots per clone at the end of the experiment (27 November to 5 December 2006). Half way through the experiment (15 November 2006) one pot of each clone was destroyed in order to measure the area of each leaf of each primary stalk or tiller identified in the same manner as for the intact leaf counts. The area of each leaf was measured with a video planimeter. Multilinear equations for estimating the area of individual intact leaves were obtained by fitting measured leaf area, as the dependent variable, to leaf position and total number of leaves per shoot, as independent variables. These equations were applied to the non-destructive leaf counts to estimate leaf area per pot at the start and end of the experiment.

Rotation experiment 2 – photosynthesis measurements

This experiment was planned to again compare clones in regard to rates of photosynthesis but at a later stage of development, during the rapid stalk elongation phase. Plants potted at the same time and manner as those used for the first rotation experiment were grown outside under regular irrigation until 20 December 2006. They were then placed in the TPF for a clone x irrigation experiment which required regular destructive sampling by cutting plants at ground level. After a sampling in early February 2007,

pots of each clone were allowed to ratoon outside the TPF with adequate water and nutrients. Rust infection developed on Q117 and KQ97-2599 but not on the other two clones which were then transferred to the TPF for a second rotation experiment in June 2007, when eight pots of each clone were rotated between the two chambers ten times.

At the end of the experiment on 4 July 07, all green leaf blades were removed and weighed from two pots of each clone. The area and dry mass of leaves from two shoots per pot were determined and the leaf area per pot derived. There was no similar measurement made at the start of the experiment and it was assumed that the relative difference between the clones was constant throughout the short experiment.

Irrigation x clones experiment (experiment 3)

On 20 December 06 when plants had produced about nine internodes, four pots of each clone were harvested and 12 pots of each clone were transferred from the outside to each of two chambers in the TPF. The pots were arranged in four rows of 12 about 1.0 m apart. An additional two pots were placed at the end of each row to ensure that all experimental plants had similar shading. Pots were mounted on mobile stands about 10 mm from ground level. The four cultivars were arranged randomly and pots were redistributed to retain the original spacing after some were removed for destructive sampling.

While outside, all pots were watered to capacity three times daily until 21 December 2006 when pots in the dry chamber had their water supply decreased. The water regimes, photosynthesis and plant extension rate measurements in the TPF were similar to those for the year 1 experiment. Four pots of each clone were sampled on 20 December 06 before irrigation treatments were applied, and four pots of each clone and each irrigation treatment were sampled on 6-8 February, 20-22 March and 1-3 May 07. Plants were processed as in year 1.

Statistical analysis

The statistical model for analysis of variance for the two rotation experiments was:

$$Y_{JKLM} = \mu + B_J + C_K + G_L + C_K G_L + R_M B_J + E_{JKLM}$$

where Y is the response variable, μ = grand mean, B_J is the effect due to the J th block, C_K is the effect due to the K th clone, G_L is the effect due to the L th glasshouse compartment, $R_M B_J$ is the M th round (incomplete block) of the J th block and E is the residual. The statistical model for analysis of variance for the clone x irrigation experiment was:

$$Y_{JK} = \mu + C_J + W_K + C_J W_K + E_{JK}$$

where Y is the response variable, μ = grand mean, C_J is the effect due to the J th clone $J=1,2,3,4$; W_K is the effect due to the K th water regime, $C_J W_K$ is the effect due to any interaction between the J th level of the clone factor and K th level of the water regime and E is the residual.

Year 3 (2007//08) – Appendix 3

By year 3 results from the project indicated that there was no difference between low and high sucrose clones in the limit to which sucrose can be stored in stalk parenchyma, though clearly there is a limit somewhere above 67% sucrose in stalk dry matter (Inman-Bamber *et al.* 2009). Differences between high and low sucrose clones were found in their responsiveness to water stress which reduces expansive growth more than photosynthesis thus altering the source-sink balance to a lesser extent in low than in high sucrose clones. Differences in high- and low sucrose clones were also found in the morphology of plants such that high sucrose clones produced fewer stalks than low sucrose clones. Low sucrose clones allocated more dry matter to leaves than high sucrose clones possibly to maintain a minimum leaf area for each stalk (Inman-Bamber *et al.* 2009).

The next experiment (year 3) was conducted to determine if the above hypothesis stands when using temperature rather than water stress to perturb the source-sink balance. We also applied a thinning treatment to test the proposal that sucrose accumulation was lower in clones with high rather than low stalk populations.

The research was conducted in the TPF as in years 1 and 2 with instrumentation and plant management conducted in a similar manner. Two temperature regimes were applied (Table 2), one typical of winter conditions and the other of summer/autumn conditions found in the Burdekin which is the largest cane growing region in Australia. The high summer temperature could not be maintained because plants would have reached roof height (6 m) before the end of the experiment.

Table 2. Temperature settings for eight selected hours in two chambers of the TPF for three periods during the experiment. The control system interpolates between a maximum of eight selected diurnal settings.

Hour	Chamber 1 (Cool)		Chamber 2 (Hot)		
	6Dec-1May	Hour	6-20Dec	20Dec-11Jan	11Jan to 1May
0	16	3	24	22	20
6	14	8	26	24	23
8	17	10	29	27	26
10	21	12	30	28	27
12	25	13	31	29	28
15	23	16	29	27	25
18	20	19	27	25	23
20	18	23	25	23	21

Clones used were KQ97-2599 and KQ97-2835 representing low sucrose types and Q117 and KQ97-5080 representing high sucrose types, as for the year 2 experiments.

More than 250 one eyed sets of each of the four clones were planted in trays on 24 May 07 and then transplanted into 27 L pots filled with 25 kg of a commercial potting mix (Bedrock Landscape supplies, Townsville, Australia) during August 2007. Tillers were removed from half the pots on 7 November 07 to leave six healthy shoots in each if possible but some pots of some clones had fewer than 6 healthy shoots at this stage. Treatments were therefore; two temperature regimes, *hot* and *cool*; four clones (KQ97-2599 and KQ97-2835 as low sucrose and Q117 and KQ97-5080 as high sucrose clones) and two thinning treatments, *thinned* and *not-thinned*. There were three replications.

Three pots with unlimited tillers per clone were removed for the first sampling on 12 November 07. All above ground plant material was removed and weighed. Six representative stalks were selected and made into pairs and then one stalk in each pair was taken at random for more detailed analysis as described previously.

On 6 December 07 pots were transferred to the chambers arranged as they were outside. On 13 December shade screens were placed along the N and S walls of each chamber in order to limit and equalize radiation that may be entering these side walls even though the sun was overhead at midday at this time of year. Alidicarb was applied to each pot on 14 December and irrigation was reduced from 3 to 1 minute to avoid drainage from the pots until it was certain that the insecticide had been taken up by the roots or was broken down.

On 17 to 18 December three pots of each clone and each thinning treatment were sampled from those pots left outside the TPF. The sample was split into large and small stalks after weighing all cut material. Large stalks were placed in three groups based on length and a representative stalk was removed from each group for sub-sampling.

During 9 to 11 January 08 pots were removed from both hot and cool chambers in the TPF, from each clone, thinning treatment and replication for the third destructive plant sampling. The remaining pots were redistributed to ensure an even spacing between them. All cut material was separated into large and small stalks or shoots and three stalks were selected from the large stalks to fully represent the large stalks, as for the second sampling. The fourth destructive sample was carried out on 17-19 March 08 in a similar manner to the third sampling.

Year 4 (2008//09) – Appendix 4

Project work on sucrose accumulation conducted in years 1 to 3 offered a fresh challenge to conventional molecular approaches which have focussed mainly on the tissue in which sucrose accumulates. Projects aimed at identifying genes involved in up or down regulation of sucrose accumulation should at least have an eye on whole plant processes to ensure that causes of sucrose accumulation rather than the effects of some other cause are being considered (Inman-Bamber *et al.* 2009). The internode sucrose accumulation model built by Dr Singels offered a framework to link knowledge of the sucrose accumulation process across different levels of organisation, from plant organs to plants cells and molecules. The model explains sucrose accumulation by maintaining the mass balance of carbohydrate generation (photosynthesis) and distribution to the various plant components and to each internode. We believe that this framework provides the best chance for a comprehensive understanding of the complex interactions between the climatic and cell environments in the production of sucrose. However the model was built and validated only on four sugarcane clones selected to represent the range in sucrose content in the Australian genepool. The experiment conducted in the final year challenged the essential components of this and other models by considering four times as many clones grown in two temperature regimes.

The 16 clones were selected for this glasshouse (TPF) experiment in a similar manner to the selection of four clones as described earlier. Eight clones at each end of the sucrose content range were selected provided they appeared to be vigorous during the plant and ratoon crops of the propagation trial.

The propagation trial was used to supply more than 250 1-eyed setts per clone which were planted in a peat/vermiculite mix during August 2007. Germinated setts were transplanted into 27 L pots filled with 25 kg of a commercial potting mix on 19-22 November 07. From January 2008, pots were inspected regularly so that excess tillers could be removed when they were large enough to grip by hand leaving six of the largest shoots per pot to develop normally. Four replicates of each clone were transferred to each of two chambers in the TPF on 8-11 April 08. A week later (14-17 April) another 64 pots were destroyed to obtain detailed data on plant biomass and dry matter partitioning to leaves, sheaths and internodes as described earlier. On 21 April 08 shade cloth was hung alongside chamber walls facing north and south to help equalize the amount of radiation entering each chamber.

All pots were harvested, one replication at a time (2-days) from 30 June to 14 July 08. The harvesting and sampling procedure was similar to the one described for the year 3 experiment apart from changes required to accommodate flowering stalks in some clones. All stalks were topped at the base of the 3rd leaf, counting as leaf 1 the flag leaf in flowered stalks and the youngest mature (top visible dewlap, TVD) leaf in non flowered stalks. Side-shoots had developed on some flowered stalks and these were added to the 'flower' component since they arose as a result of the flowering phenomenon. Side-shoots were rated 1 to 5 from small to large the largest being about 700 mm from the base to the TVD.

Model of sucrose accumulation in sugarcane internodes – Appendix 5

Dr Abraham Singles visited the Davies lab in Townsville for six months in 2008 to build a processes level model of dry matter partitioning to internodes of sugarcane. The visit was funded partly by a grant from the Science Team at CSIRO's corporate division. A simulation model of source-sink processes and biomass partitioning at a phytomer (leaf and associated internode) level could provide a useful link between cell level biochemical models and crop level growth models. This could advance understanding of genetic and environmental control of sucrose accumulation.

A framework for aboveground biomass partitioning between competing sinks (leaf growth, stalk structural growth and stalk sugar storage) was proposed where partitioning depends on temperature, water status and on the physiological age of the phytomer. Also proposed was that these relationships are strongly dependent on genotype through the structural demands (sinks) imposed by the phenological development of leaves and tillers. This hypothesis was translated into a mathematical model and then tested by comparing simulations with observations from the glasshouse experiments conducted in years 2 and 3 as reported above.

Daily above-ground assimilation of CO₂ was simulated using the radiation use efficiency (RUE) approach, where intercepted radiation is calculated from leaf area (interpolated from measurements) using Beer's law. RUE was taken as a product of a genetically determined maximum value (estimated from year 2 data), and zero to unity control factors for temperature (derived from Liu and Bull 2001) and for water status (calibrated on year 2 data).

Whole plant partition fractions for structural sinks were calculated using a cultivar specific reference value (at optimal temperature and water status) and a cultivar specific responses to water status and temperature. The cultivar reference values for stalk and leaf structure partitioning were estimated from experimental data and trends. For example, high sucrose (HS) clones partitioned less to leaf than low sucrose (LS) clones, while partitioning to stalk structure did not differ between clones. Temperature control functions were derived from the literature (Robertson *et al.* 1998; Inman-Bamber 1994) while water status functions were calibrated using year 2 data.

Sugar storage in the stalk was calculated as the difference between net assimilation and leaf and stalk structural growth. Whole plant hexoses to total sugars ratio was calculated assuming a linear relationship with the ratio of structural (stalk and leaf) mass to total mass derived from year 2 data.

Whole plant daily increments of the various pools were divided between the primary shoot and all the other shoots based on the measured ratio in biomass between primary and secondary shoots. Daily primary shoot increments were distributed to individual internodes according to carbon demands for leaf and stalk expansion (cell wall elongation) and stalk densification (cell wall thickening) based on the physiological age of internodes (Rae *et al.* 2005; Lingle 1999). Carbon demands for these processes were calculated using a Weibull function, which was then normalised relative to the total calculated demand for the stalk.

Distribution of sugar storage to internodes was calculated from storage capacity and current sucrose content. Storage capacity was calculated from the genetic maximum (taken as the highest observed sugar to stalk fibre ratio measured in year 2), and current fibre mass and physiological age. Maximum sugar storage capacity was assumed to be independent of genetic and environmental factors. Whole plant hexoses mass was allocated to internodes in proportion to the relative respiration demands of each internode (Bindon and Botha 2002), with upper and lower bounds of hexoses to sugars ratios of 0.85 and 0.03, respectively.

Field trials on GxE for lodging

Four replicated field experiments were conducted with the aim of assessing the relative contributions of genotype (G) and environment (E) to the GxE interaction and to determine if irrigation can be used to delay lodging without limiting yield.

Lodging experiment 1 - Appendix 6.

A trial was planted at Kalamia Estate near Ayr in September 2005 to determine how to reduce lodging while achieving high yields. The concept was to gain control over plant height through irrigation, reducing plant height before the wet season to reduce lodging. Other plots would be irrigated liberally to encourage lodging and then both lodged and erect treatments would be allowed to grow rapidly with irrigation to determine the extent to which the shorter and more erect treatments can catch up or even overtake the lodged ones.

The treatments were designed to encourage or discourage lodging without causing excessive water stress. Auxanometers were installed to monitor plant extension rate (PER) as a means of assessing the degree of water stress sensed by the plants. Irrigation treatments were based on frequency rather than amount, the amount being limited to what could be applied before water started to pond and then run off the plots.

Irrigation was applied to plots in the following manner after 7 February 06. The last irrigation (furrow) was applied to all plots on 20 December 05.

W1 = frequent irrigation (every week day)

W2 = less frequent (twice a week)

W3 = W4 = not irrigated

The whole trial was lodged extensively in June 2006 assisted by cyclone Larry in March 2006 and it was not possible continue with the planned sampling procedure.

Lodging experiment 2 - Appendix 7.

Lodging experiment 1 was harvested on 26 July 06 and was allowed to ratoon with normal estate management of weeds, nutrients and irrigation before imposing irrigation treatments on 7 November 06. Irrigation treatments were monitored carefully using PER measurements to ensure stalk heights between 50 and 100% of potential prior to the wet season. Lodging was rated on four occasions and a full destructive plant sampling was conducted on 7 March 07. Extensive lodging in June in all plots made it impossible to assess the expected recovery of cane in treatments that we were hoping would remain erect after limiting irrigation.

Lodging experiment 3 - Appendix 8.

This experiment was designed to assess whether irrigation management favours certain clones in selection trials more than others. It is possible that clones prone to lodging will be selected preferentially when irrigation is used sparingly because lodging is delayed compared to a fully irrigated and more commercial situation. The experiment was also designed to assess the capability for erect but retarded crops to catch up with more advanced but lodged crops after the wet season. Clones that are more prone to lodging should benefit more from the delayed growth pattern.

Treatments were:

Cultivars

- a. High lodging X 2
- b. Low lodging X 2

Irrigation regimes:

- a. Frequent irrigation to encourage stalk elongation and lodging (W1)
- b. Infrequent irrigation to delay stalk elongation and lodging (W2)

The design was a split plot with three replications; whole plots for irrigation treatments and sub-plots for clones.

Choice of clones for the experiment was based on lodging ratings reported in Appendix 9 and on the quality of seed cane available in the propagation plots. KQ97-4299 and KQ97-4606 ranked third as the most erect clones in at least one of the lodging assessments of the propagation plots and MQ77-340 ranked fourth and KQ97-2782 second as the most lodged of the 34 clones and cultivars.

Seed cane was cut from the propagation plots on 10 July 06 and many buds were swollen on lodging types KQ97-2387 and MQ77-340 which also had long internodes hence fewer buds. Seed material was selected carefully to ensure that no more than two eyes were missing or damaged per 2 m stalk. The trial was machine planted, two setts at time on 11 July 06. When leaves were about 30-40 cm high (5-6 leaves) on 13 September 06, the three net rows had gaps filled by transplanting stools from guard areas particularly for KQ97-2782. Auxanometers were attached to five plants each of two clones and two irrigation treatments on 18 November 06 to monitor plant growth.

At the end of the wet season differences in stalk height and lodging between irrigation treatments were insufficient to test the hypothesis that erect crops are able to catch up with lodged crops after the wet season. Lodging differences between clones were significant but the clones did not interact with the irrigation treatment as was hoped. It was decided to harvest the trial as early as possible in June in order to allow sufficient time in the ratoon crop, to establish height and lodging differences before the next wet season.

Lodging experiment 4 - Appendix 10.

This was repeat of experiment 3 and was conducted on the first ratoon crop after harvesting the plant crop on 30 June 07. The whole site was flood irrigated on 15 July 2, 27 September and 15 October 07. From mid-October to mid-April, wet and dry treatments were irrigated independently using WaterSense which calculates a number of attributes of the soil water-plant-atmosphere continuum including soil water deficit, demand for water by the atmosphere and supply of water by the roots. The wet treatment was irrigated when the soil water deficit was about 60 mm as determined by WaterSense and the dry treatment was irrigated when the soil dried out such that only 50-75% of the daily water requirement could be supplied by the roots. Irrigation was applied to each treatment independently through a trickle system fitted with flow meters. Two lines of emitter tape delivered 24 mm per irrigation to wet plots and one line of tape delivered half that amount to dry plots. No runoff was observed over the 8 hours required to deliver these amounts. The trickle tape was removed after mid-April and all plots were furrow irrigated when the soil water deficit reached 60 mm as for the wet (unlimited irrigation) treatment.

The wet treatment received a total of 1152 mm irrigation plus 1285 mm rain and the dry treatment 727 mm irrigation plus the 1285 mm rain (Fig. 2).

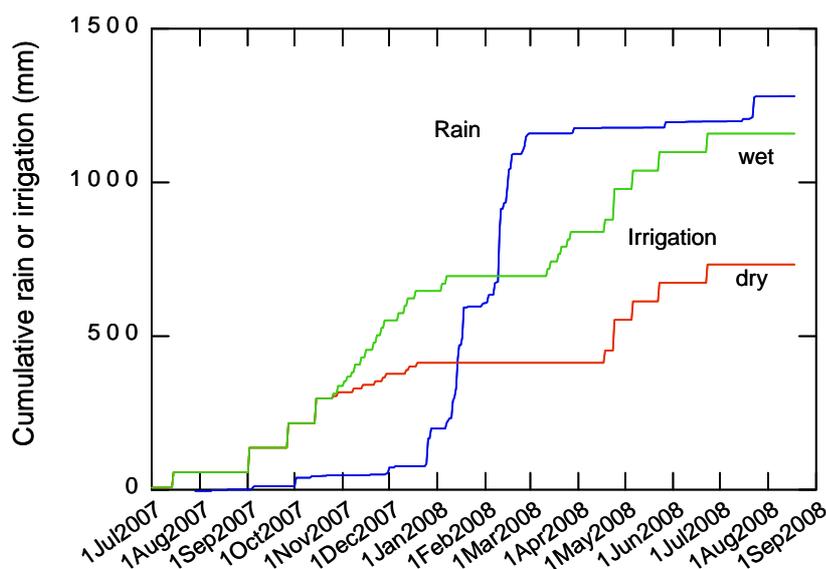


Fig. 2. Cumulative rainfall and net irrigation for the duration of the fourth lodging experiment

Plots were rated regularly for stalk height, lodging angle, canopy development and flowering. Lodging was judged by the angle from the vertical of stalks at the base. This was often variable within a plot so stalks were grouped mentally into categories which were rated separately and a weighted average obtained.

Sample harvesting was conducted on two occasions, once after the end of the irrigation treatment period (15 October 07 to 3 April 08) and on 18-20 August 08, shortly before machine harvesting. Ten adjacent stalks were cut at the base and bundled before removing all plant material from 16.2 m² of net plot area. All material was weighed and the 10 stalk sample was then partitioned into leaf blades and sheaths and stalks while counting green leaves and internodes. Dry matter content was determined for each part and sucrose content for the stalks, following the method described by Muchow *et al.* (1993).

Evaluation and reporting

State of sugarcane physiology knowledge – Appendix 12

The international workshop on sugarcane physiology held in 2003 provided the stimulus for many scientists involved in sugarcane improvement and management at various levels of organisation, to cross organisational boundaries. Chief investigators of this project were asked to be guest-editors of papers delivered to the workshop, for a special issue of *Field Crops Research* devoted sugarcane physiology. The special issue (vol 92, 2005) combined 18 high quality papers on various aspect of sugarcane physiology including all levels of organisation between molecular and mill catchment levels. This publication serves as a benchmark for the state on knowledge of sugarcane physiology at the various levels of organisation as well as a measure of the extent to which knowledge was spanning these levels at the time of publication. The special issue was summarized in a paper by chief investigators and others as presented in Appendix 12.

Knowledge, Attitudes, Skills and Aspirations of key members of the BSES-CSIRO-CSR Sugarcane Variety Improvement Program – Appendix 13

A questionnaire was devised with the help of Drs Mac Hogarth and Emma Jakku (social scientist) to document the Knowledge, Attitudes, Skills and Aspirations (KASA) of key plant breeders working in the BSES-CSIRO-CSR Sugarcane Variety Improvement Program. The questionnaire was completed by 11 people employed presently, or in the recent past, in the Australian breeding program. Nine of the questionnaire respondents were directly involved in daily plant breeding activities.

Selected respondents were guided through the questions during semi-structured interviews conducted by Drs Sarah Park and Geoff Inman-Bamber. The questions were devised to elicit breeder's KASA in regard to incorporating physiological understanding into breeding procedures. Physiological understanding was taken to be a mechanistic knowledge of how the plant works, for example to store sucrose instead of using it for growth. The questions firstly used a simple rating system to capture answers. More open-ended questions were then used to elicit greater understanding of the responses. The interviews were recorded to provide an accurate record of the discussion to serve as the basis for summary and analysis. The recordings and their transcriptions are to remain completely confidential and the respondents are not personally identified.

Key workshops and meetings – Appendix 14

Workshops were held each year of the project in August to November to report and evaluate the progress of the project. Other workshops and seminars were arranged that proved extremely useful in promoting the aims of the project.

	Date	Place	Event
1.	10 Aug. 05	Townsville	Workshop and steering panel
2.	17 Oct. 06	Townsville	Workshop and steering panel
3.	16 Oct. 07	Townsville	Workshop and steering panel
4.	1 Oct. 08	-	Milestone 8. Interim evaluation of sub-traits
5.	7 Oct. 08	Brisbane	Mini-workshop on sucrose accumulation
6.	26-27 Nov. 08	Townsville	Workshop and steering panel
7.	5 Mar. 09	Durban	Mini-workshop on molecular to whole plant knowledge of sucrose accumulation
8.	1 April 09	-	Milestone 9. Contribution of project to breeding technology evaluated against baseline

OUTPUTS

Outputs from the project are recorded in the conclusions of the journal papers which are in various stages of publication. Outputs are numbered and reported here under year headings for clarity and links to the various papers should also be clear from citation of the relevant appendices. Six refereed conference papers were produced in addition to the journal papers and outputs recorded in these will be summarised where appropriate to add to demonstrated outputs from this project.

Glasshouse research on sucrose accumulation

Year 1 (2005//06) – from Appendix 1

1. *New concepts for Leaf area development*

Previously published data for area per leaf indicate that the area of each successive leaf increases up to about leaf 15 and then stabilizes (Robertson *et al.* 1998). The results from the first sampling in our experiment indicated that maximum leaf area (about 380 cm²) had been reached at leaf 10 which was roughly equivalent to leaf 15 in Robertson's experiment because we collected leaves attached to only above-ground nodes. Once plants of Q138 and Q183 were in the TPF in our experiment, leaf size increased to about 700 cm² which exceeded the maximum leaf area for Q117 of 600 cm² obtained in a controlled environment facility (CEF) and 400 cm² for Q117 in the field (Robertson *et al.* 1998). It appears therefore that environment has a large influence on the leaf area profile of sugarcane plants. Singels *et al.* (2005) proposed a conceptual model for leaf development in which they assume that each leaf has a genetically determined heat sum (thermal time) during which to develop. The final area of the expanding leaf depends on this heat sum 'window' which increases with each successive leaf, as well as the source strength (size and activity) of older leaves. Increased area of successive leaves may require an even greater increase in the supply of photo-assimilate to these leaves during development because leaf area per unit dry mass decreases with successive leaves at least after 20 have appeared (Robertson *et al.* 1998; Inman-Bamber 2004). The number of green leaves per stalk in the TPF detailed in our study was much greater than usually observed in the field (Inman-Bamber 2004) probably because of the lack of shading of lower leaves in the TPF. Thus source strength per stalk in the TPF could have been greater than in the field producing these large leaves. The maximum number of mature green leaves in the field is normally about 8 to 10 per stalk (Robertson *et al.* 1998; Inman-Bamber 2004) compared with the maximums of 14 for Q138 and 19 for Q183 in the TPF.

2. *Photosynthesis reflects biomass gain and PER gain in top mass.*

Water stress reduced total biomass gain by 143 g per pot (19%) and it reduced biomass gain in leaves plus cabbage (tops) by 84 g (37%), in stalks by 68 g (14%) and it increased sucrose mass gain by 41 g (27%). Water stress reduced whole plant photosynthesis by 18% thus largely accounting for the 19% reduction in biomass accumulation and it reduced PER by 41 % thus largely accounting for the 37% reduction in mass of tops.

3. *Hypothesis of Singels et al. (2005) refined.*

In steady state (wet and hot) conditions sucrose content (SCd) of whole stalks increased over time because of the accumulation of more mature internodes in which SCd appeared to reach a maximum of about 420 mg/g in *wet* stalks and 470 mg/g in *dry* stalks. Under moderate water stress SCd increased most in internodes 8 to 11 giving rise to a steeper downward gradient in SCd toward the top of the stalk. To this extent the hypothesis of Singels *et al.* (2005) was supported by our data but the notion that the response to ripening stimuli occurs only at the top of the stalk was not supported. Large seasonal changes (30%) in the sucrose mass of fully expanded basal sections of stalk were reported by Inman-Bamber *et al.* (2002) illustrating the large capacity for sucrose storage and reuse of assimilate in mature internodes with SCd >420 mg/g.

4. *Whole plant photosynthesis is about half that of the youngest mature leaf*

For the *wet* treatment, projected mean photosynthesis per unit leaf area for the whole plant (P_A) at $S = 1500 \mu\text{mol}/\text{m}^2/\text{s}$ was $17.9 \mu\text{mol}/\text{m}^2/\text{s}$ which is less than half the photosynthetic rate of young leaves

exposed to the same radiation in the cuvette of the Li6400. No doubt P for lower leaves was reduced because of some shading as well as general aging as was evident in wheat where P decreased linearly with age about 3 days after the ligule appeared (Rawson *et al.* 1983). A similar result was obtained for sugarcane where P increased initially between 7 and 14 days of leaf emergence and then declined from a maximum of 30 $\mu\text{mol}/\text{m}^2/\text{s}$ to half that rate 31 days later (Vu *et al.* 2006).

5. *Photosynthesis continues at 50% when leaf extension stops*

In our experiment P for single leaves was as high as 45 $\mu\text{mol}/\text{m}^2/\text{s}$ on 16 March and was reduced only to 22 $\mu\text{mol}/\text{m}^2/\text{s}$ when PER was reduced to near zero for lack of water. This is consistent with results of a rainout shelter experiment where stomatal conductance was reduced only 50% when PER reached zero (Inman-Bamber *et al.* 1995).

6. *Sugarcane may have a natural ripening process.*

We demonstrated under steady state conditions that expansive growth slows down before photosynthesis even while water supply and temperature remain regular or constant.

7. *Reduced expansive growth without a matching reduction in photosynthesis increases sucrose yield*

The experiment proved that reduced expansive growth without a matching reduction in photosynthesis would increase sucrose yield as well as SCd at least while SCd was less than published ceiling values (up to 600 mg/g, Berding 1997). Since such ceiling values are seldom obtained in the field (Inman-Bamber *et al.* 2002) it is proposed that irrigation could be applied so as to regulate expansive growth down by about 30% throughout the crop cycle without an equivalent reduction in photosynthesis or biomass accumulation, leading to higher SCd and higher sucrose yield in commercial crops. The additional sucrose yield would come from photo-assimilate that would otherwise have been used to produce additional leaf and stalk tissue as was the case in the TPF experiment. It is not known how hard this would be to achieve in practice and rainfall would of course limit the option for regulating expansive growth through irrigation however the benefits are attractive both for increased SCd and possibly sucrose yield as well as for reduced water use.

8. *Techniques developed to control the balance between growth and storage.*

The techniques developed in year 1 to control PER and measure the resulting changes in carbon partitioning now allow further examination of both the control of the balance between growth and storage and the extent of genotypic variation to the response of reduced PER.

Year 2 (2006//07) – from Appendix 2

9. *Photosynthesis is similar in high and low sucrose clones.*

Biomass accumulation was remarkably similar in three out of the four clones despite significant differences in photosynthesis per pot during the first rotation experiment. However the second rotation experiment indicated that clonal differences in photosynthesis per pot of larger plants were small. From this we concluded that differences in sucrose accumulation between high- and low sucrose clones was not due to large differences in the rate of photosynthesis.

10. *Greater sink strength in low sucrose clones did not increase photosynthesis.*

Fresh cane mass per pot differed little between clones and since dry matter content was greater in high than in low sucrose clones the mass of tissue yet to be 'filled' with photo-assimilate was greater for the low than the high sucrose clones. This greater sink strength did not favour increased dry matter accumulation or photosynthesis in the low sucrose clones which is counter to expectations from the work of McCormick *et al.* (2008) who proposed that photosynthesis is limited when high levels of sucrose accumulate in the stalk.

11. Variation in stalk population is related to variation in sucrose content

The clones differed substantially in stalk number and in mass per stalk. The large number of shoots per pot in KQ97-2835 was associated with high fraction of dry matter in tops, lower stalk dry mass and shorter stalks compared to Q117 and KQ97-5080. While biomass accumulation in KQ97-2835, Q117 and KQ97-5080 was similar and was slightly lower in KQ97-2599, photo-assimilate in KQ97-2835 and KQ97-2599 was distributed among about 9-11 stalks per pot for KQ97-2835 and 7-10 stalks per pot for KQ97-2599 compared to only 4-7 stalks for Q117 and KQ97-5080. The most important aspect of stalk numbers was manifest in the large mass of leaf relative to stalk in the high population (low sucrose) clones compared to the low population (high sucrose) clones. These leaves did not result in additional photosynthesis. It is suggested that the relatively large amount of new leaf tissue produced by the high population, high sucrose clones placed an additional demand on photo-assimilate in these clones and delayed the accumulation of sucrose in the stalk.

12. Low sucrose clones accumulate sucrose 'normally' in some internodes

Maximum dry matter content of the internodes of the two low sucrose clones was only slightly lower than for the high sucrose clones and the proportion of sucrose in this dry matter for low sucrose clones reached levels regarded as close to maximum in previous published research (Berding 1997; Inman-Bamber *et al.* 2002). The sucrose content pattern in KQ97-2835 internodes on 1 May 2007 was similar to that of Q117 internodes on 20 March 2007 (Fig. 3) and it is possible that KQ97-2835 was about eight weeks behind Q117 in allocating photo-assimilate to the stalk. If this was the case then there was no qualitative difference between these clones in regard to sucrose accumulation and the low sucrose content of KQ97-2835 was simply a matter of delay caused by the need to supply more developing leaf tissue with photosynthate.

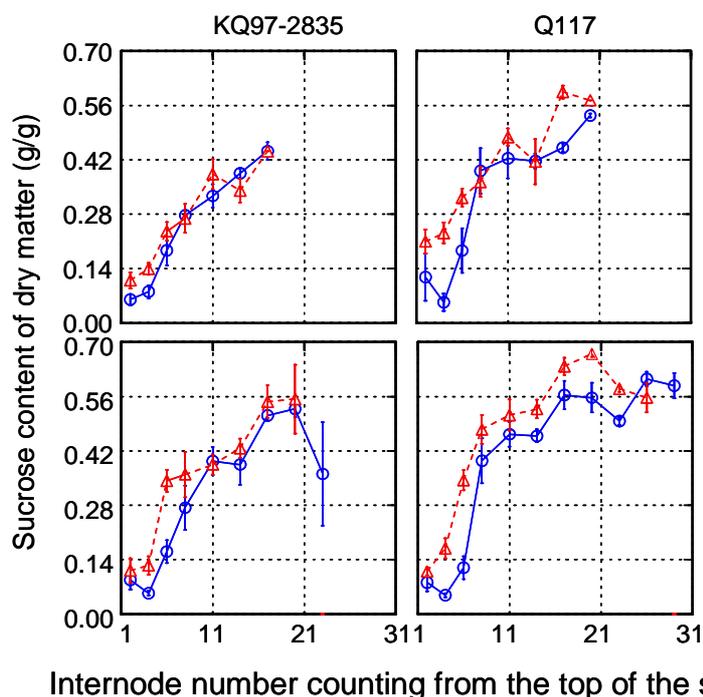


Fig. 3. Sucrose content of dry mass determined on 21 March and 1 May 2007 for internodes of two clones grown with minimal (○) and moderate (△) water stress. Bars are 2 x standard error of the mean.

13. Low sucrose clones can accumulate high levels of sucrose in some internodes.

It is remarkable that two clones chosen because of their low sucrose content should be able to accumulate sucrose equivalent to a highly selected clone (cultivar) like Q117, albeit in only a limited number of basal internodes. As the tested clones were related it suggests the maximum amount of sucrose that can accumulate in stalk tissue is not the limiting step for sucrose accumulation and that genetic differences, at least in this background, reside more in the morphology of the plant and the way this influences supply and demand for photo-assimilate.

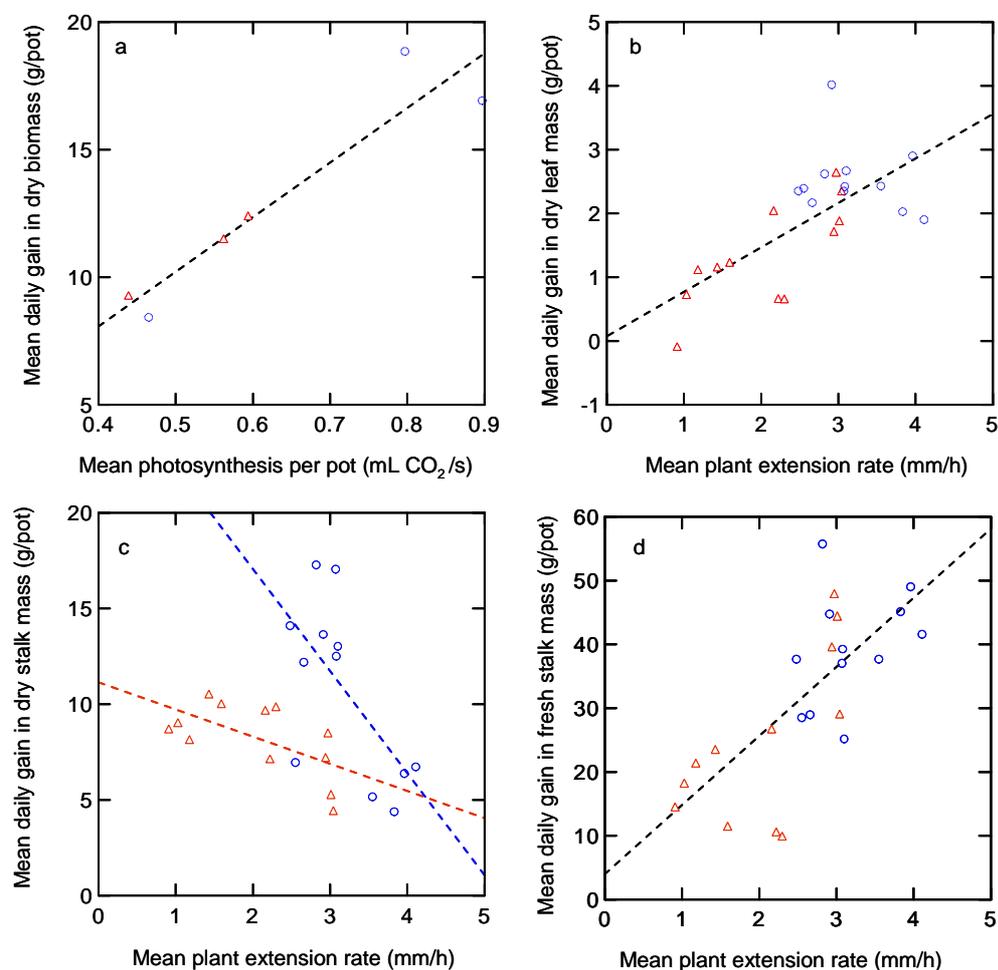
14. Model for sucrose accumulation

A conceptual model for sink demand on photosynthesis can be suggested from the relationships in Fig. 4. Daily biomass gain (B) which can be estimated from photosynthesis per pot (P), is assumed to be split equally between the number of stalks per pot (N). Sink strength of leaves as reflected in dry mass gain of green leaves is associated significantly with plant extension rate (E). The demand for new fibre (cell wall) in the stalk would be also associated with extension rate. Thus, if sucrose (S) is stored when supply of assimilate exceeds demand by new leaves and structural stalk tissue, then storage rate (dS/dt) should be related to P , E and N as:

$$dS/dt = (20.6P(a+bE))/N$$

where $t = 1$ day and $20.6P$ comes from the data in Fig. 4. This equation with $a = 4.0 \pm 0.52$ and $b = -0.67 \pm 0.19$ fitted by least squares, accounted for 72% of the variation in gain in mass of sucrose per pot per day when applied to the 24 observations derived from 3 sampling intervals, 4 clones and 2 water regimes. This simple concept emphasises the role of stalk numbers and the response of expansive

growth relative to photosynthesis as an explanation for variation in sucrose accumulation rate between clones. Many more clones need to be investigated before the role of these sub-traits can be established with certainty. If it proves to be valid then the difference between high- and low sucrose clones may well be explained purely on source-sink attributes as represented in this model and the biochemistry of tissue which is up- or down regulated for parts of the sucrose accumulation pathway (Rae *et al.* 2005;



Grof *et al.* 2007) may be an effect rather than a cause of the difference.

Fig. 4. Mean daily gain in dry biomass and mean photosynthesis per pot for each sampling interval and treatment (a) and mean daily gain in dry leaf mass (b), dry stalk mass (c) and fresh stalk mass (d) versus mean plant extension rate for each clone, sampling interval and treatment (\circ =*wet*, \triangle =*dry*). $Y=20.6X \pm 1.4$ g/d, $r^2=0.99$, $P<0.001$ for (a), $Y=0.107 + 0.697X \pm 0.67$ g/d, $r^2=0.47$, $P<0.001$ for (b). $Y=11.1 - 1.42X \pm 1.5$ g/d, $r^2=0.37$, $P=0.035$ for the *dry* treatment in (c), $Y=27.7 - 5.34X \pm 3.7$ g/d, $r^2=0.42$, $P=0.023$ for the *wet* treatment in (c) and $Y=3.8 + 10.8X \pm 9.3$ g/d, $r^2=0.53$, $P<0.001$ for (d).

As may be expected the variation in sucrose content [S] for samplings 2, 3 and 4 was explained largely by mean photosynthesis per pot and extension rate over the preceding interval, and stalk number at the time of sampling:

$$[S] = 0.697 + 0.11P - 0.089E - 0.028N \pm 0.056 \text{ g/g } (r^2=0.83)$$

Year 3 (2007//08) – from Appendix 3

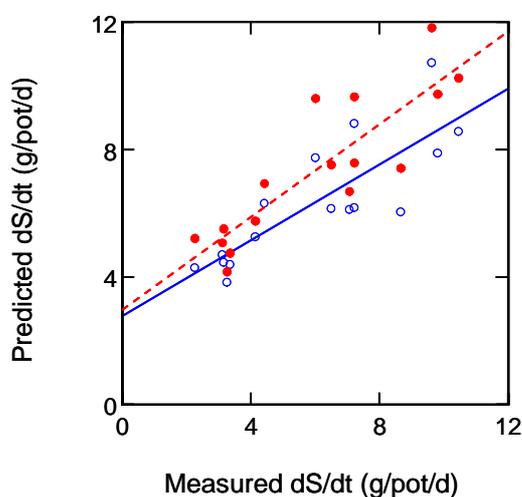
15. Temperature not as effective as water stress as a means of reducing sink strength

Temperature had no effect on photosynthesis per unit leaf area and it reduced photosynthesis per pot only through reduced leaf area. While temperature had a striking effect on PER and stalk height and a smaller but significant effect on stalk fresh mass (cane yield), it had little impact on the partitioning of dry matter between leaves and stalks. The large reduction in PER did not translate to an equally large reduction in fresh cane mass because stalks tended to thicken in cool conditions and they also increased in DM content more rapidly resulting in near equal dry matter partitioning to stalks in both temperature regimes.

16. Model developed in year 2 is valid at least for the experimental clones

The simple model of sucrose accumulation developed for the four clones in year 2 accounted for 69% of the variation in sucrose mass gain in the year 3 experiments. While the % of variation explained with independent data was only slightly lower than the 72% variation explained with dependant data, the estimates differed significantly from the observations (Fig. 5). The ranges in components of the model indicate their influence on sucrose accumulation after correcting for the deviation of the estimate from the observed sucrose accumulation. Total biomass accumulation (8.63 to 23.3 g/pot) had the greatest influence by increasing sucrose accumulation 1.70 to 2.0 times. Stalk number (4 to 8 per pot) was next with sucrose accumulation 1.30 to 1.66 times higher at the low than the high extreme. PER (1.88 to 3.49 mm/h) had the least influence with sucrose accumulation 1.20 to 1.45 times higher at the low than the high extreme.

Fig. 5. Sucrose accumulation rate (dS/dt) measured in this experiment and predicted by the model



developed in an earlier (Inman-Bamber *et al.* 2009) experiment (open symbols and solid regression line). Estimates of dS/dt with a dampened response to E (PER) are shown as open symbols and broken regression line.

17. Sub-traits for sucrose accumulation suggested

It is tempting to draw some conclusion about selecting for high rates of photosynthesis, low stalk numbers and reduced PER on the basis of these results but the model while valid for the four clones in two independent experiments may only apply to these clones. Also we showed earlier that there may be no difference in photosynthesis rates between high and low sucrose clones (Inman-Bamber *et al.* 2009).

Irvine (1975) also failed to find any association between photosynthesis per unit leaf area and sucrose content.

18. Explanation for lack of response to thinning

The hypothesis and model developed earlier indicated that stalk number was important for the distribution of dry matter between leaves and stalks and for sucrose accumulation in the stalk. The results of this experiment indicate that shoot or stalk number had very little impact on biomass accumulation, dry matter distribution or sucrose accumulation and had little impact on clonal differences. This does not necessarily destroy the hypothesis. The high population-low sucrose clones allocated more assimilate to leaves than the high sucrose- low population clones regardless of our attempts to control stalk population. This may indicate that sink strength of leaves is linked genetically to stalk numbers at least in these clones and could not be altered simply by removing stalks. Plants with inherently high stalk numbers would need to have a high priority for photo- assimilate for leaf development otherwise strong competition between individual stalks would not allow large numbers to survive.

Year 4 (2008/09) – from Appendix 4

19. Glasshouse pot technique was highly successful in determining morphological and physiological differences between the 16 clones

The glasshouse pot technique was highly successful in determining morphological and physiological differences between the 16 clones in the study since all attributes measured differed significantly ($P < 0.001$) between clones.

20. Selection for superior clones could start in potted plants

Differences between clones in the glasshouse were similar to their differences observed in the field. This raises the interesting prospect of screening sugarcane clones in pots rather than in the field at least for the early stages of selection. Thomas (1981) conducted six comparisons with 48 to 204 clones growing firstly in very small pots (560 ml) and later in the field as single stools. Total fresh biomass, stalk fresh mass and brix of field grown plants were highly correlated with similar measurements made earlier on potted plants and Thomas (1981) concluded that selections could be made from the results of potted plants grown originally for vegetative propagation only.

21. Model for sucrose accumulation could also apply to 16 clones

In the experiment with 16 clones, low sucrose clones had more stalks than high sucrose clones and expansive growth appeared to be less responsive to reduced temperature in low than in high sucrose clones. The difference in stalk height between cool and hot conditions was 66 cm for high sucrose clones and only 38 cm for low sucrose clones. The clone x temperature interaction was significant for the proportion of biomass in the stalk component and the response to reduced temperature was greater for low sucrose clones (11%) than for high sucrose clones (5%) which is opposite to what one would expect from the stalk height measurements. We showed in earlier work that leaf and stalk elongation directly reflected changes in dry matter partitioning between leaves and stalks when water stress was used to alter source- sink relations (years 1 and 2) but not when temperature was used (year 3). This was because stalks were thinner in hot than in cool conditions. It is possible that stalk diameter was reduced more in low than high sucrose clones by increased temperature thus accounting for a smaller height difference but larger difference in dry matter partitioning to the stalk for low sucrose clones in cool and hot conditions. Flowering was more extensive in low sucrose clones and this could account for such a response.

22. Credible physiological and morphological differences between sugarcane clones with different sucrose contents

The ability to distinguish easily between clones from glasshouse results ensured that the differences observed between high and low sucrose types was credible. High sucrose types had more green leaves per stalk but they allocated proportionally less biomass to leaves and leaf sheaths (cabbage) as well as to dead leaves so an additional 10% biomass could be allocated to the stalk component. Leaf area per pot was similar for high and low sucrose clones but specific leaf area was higher for low sucrose clones so the fewer leaves in these clones were thinner and larger than for high sucrose clones.

Model of sucrose accumulation in sugarcane internodes – Appendix 5

23. Internode model works well thus supporting the hypothesis that sucrose accumulates after other priorities for photo- assimilate have been met. Genes that control these priorities are at least as important as those for sucrose accumulation in storage tissue

In the year 2 experiment, low sucrose (LS) clones partitioned more assimilate to leaf than high sucrose (HS) clones in both treatments (Fig. 6). Although mean leaf size was smaller and mean leaf appearance rates slower, the higher shoot number presumably led to a bigger demand for leaf structural growth. More partitioning to leaf in LS clones resulted in less partitioning to sugar storage. Partitioning of sugars to sucrose was less, and to hexoses more, in LS clones in both treatments. The model was able to mimic these trends well, as it was semi-calibrated on the data.

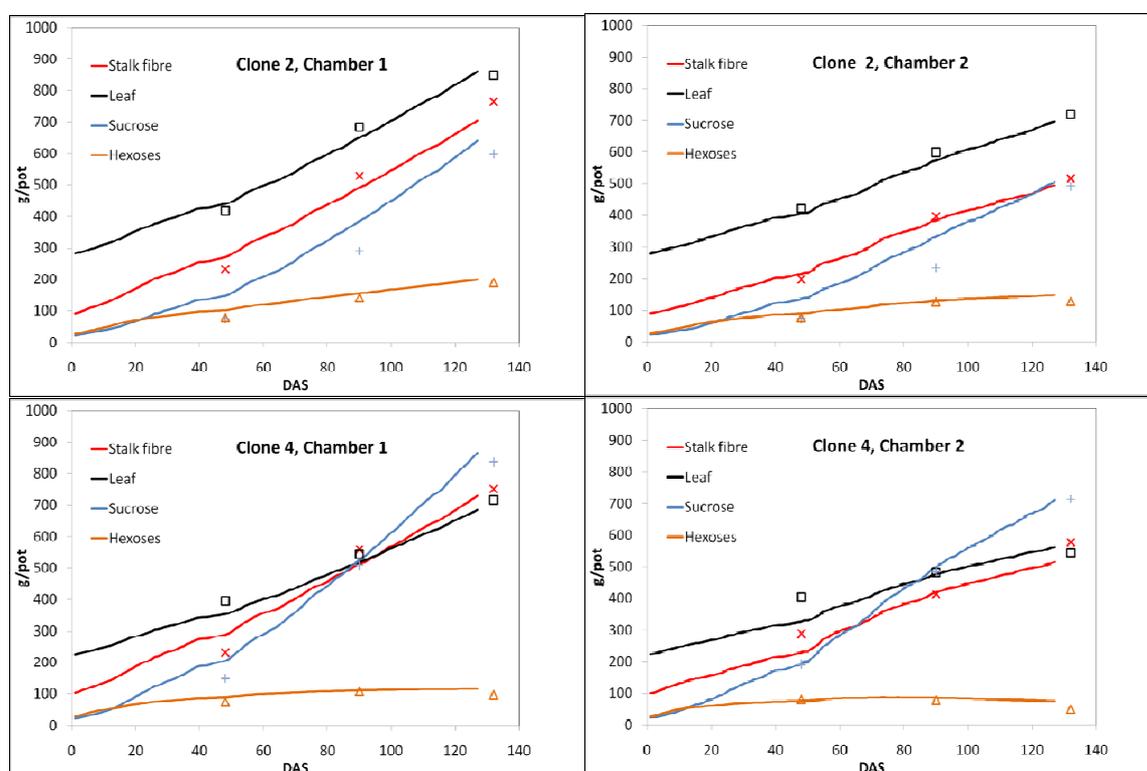


Fig. 6. Simulated (lines) and observed (symbols) leaf mass (□), stalk fibre (X), stalk sucrose (+) and stalk hexoses (△) as a function of days after the start of the treatments (DAS) for clone 2 (low sucrose) and clone 4 (high sucrose) in chamber 1 (wet) and chamber 2 (dry) in the year 2 experiment.

Simulated values of stalk fibre, sugar, sucrose and hexoses amounts per internode compared well with values observed at the final sampling of the year 2 experiment. The model mimicked the unstressed sucrose content profile of the stalk well (Fig. 7) and demonstrated the impact of faster maturity rates of HS clones, compared to LS clones. The model was unable to mimic the observed increase in sucrose content of top internodes due to water stress. The partitioning response coefficients therefore need further investigation.

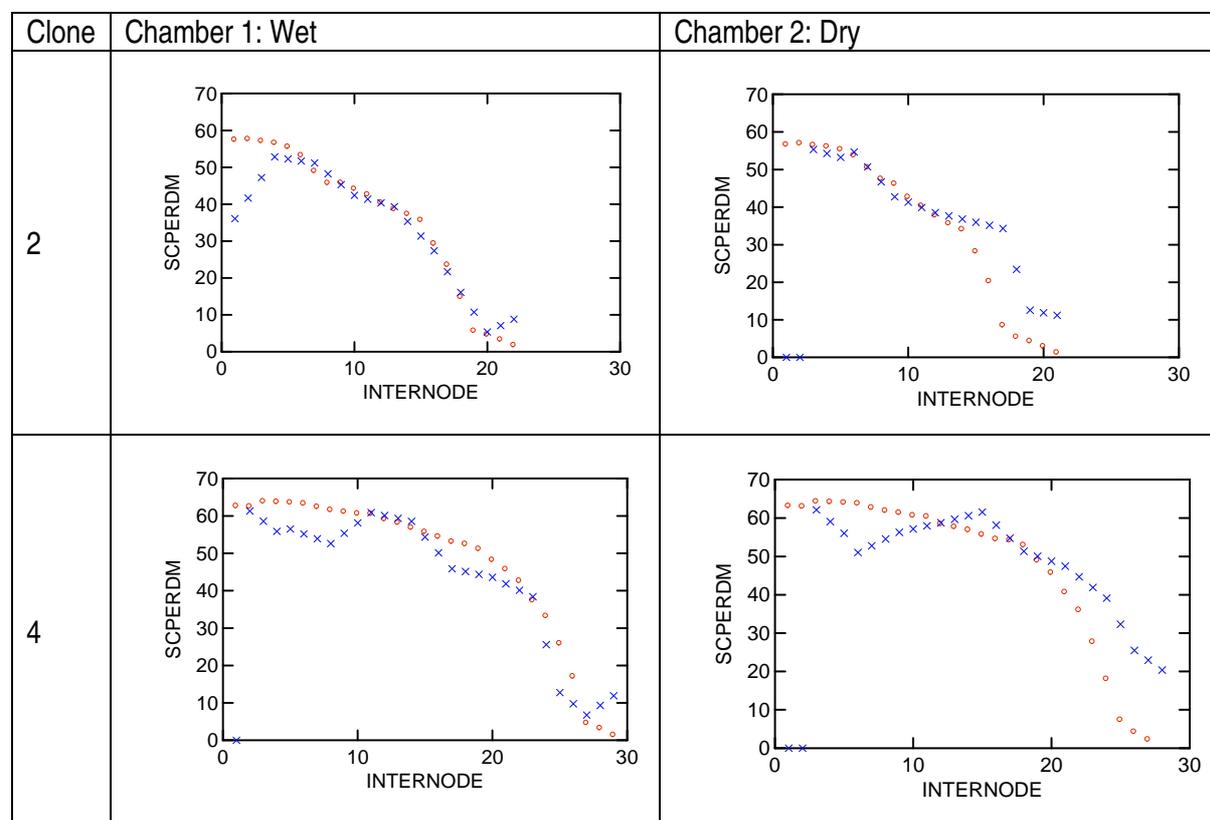


Fig. 7. Simulated (O) and observed (X) sucrose content (dry mass basis) per internode (numbered from the base of the stalk) for a low (2) and high (4) sucrose clone in the wet and dry treatments of the year 2 experiment.

Lodging

24. Lodging is controlled by manipulating plant extension rate through irrigation

In the first lodging experiment plant extension rate (PER) was greater in well watered (W1) treatment than in the other treatments during February but then dropped below PER of the other treatments during March (Appendix 6). Nevertheless differences in PER were associated with differences in lodging score taken after the wet season (Fig. 8).

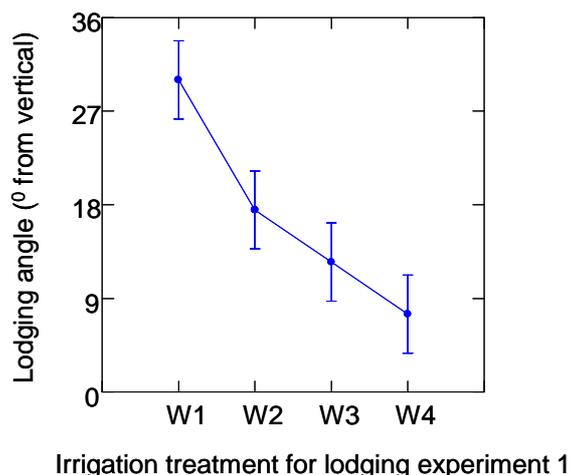


Fig. 8. Lodging rating made on 20 May 2006 for four irrigation treatments of the first lodging experiment

25. *Growth at night is not as depend on irrigation as daytime growth.*

In the second lodging experiment (Appendix 8), PER differed between treatments mainly in the middle of the day when W4 plants in particular were often growing only very slowly due to water stress while W1 plants were still growing rapidly albeit at a somewhat reduced rate compared to evening growth which was as fast as 5 mm/h (Fig. 9).

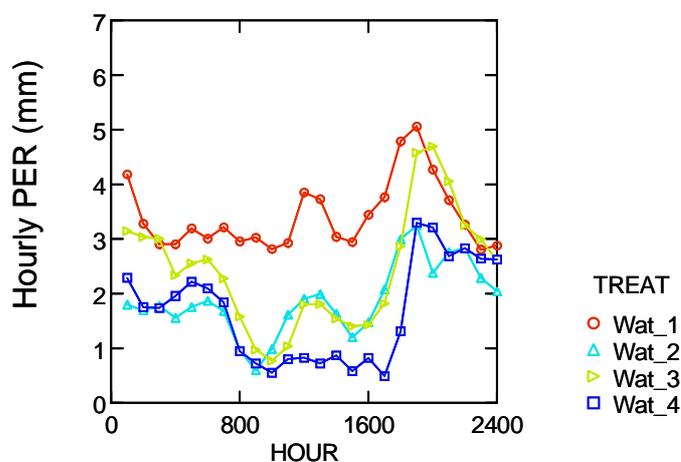


Fig. 9. Hourly PER for 23 Nov. 2006 during the second lodging experiment.

26. *Lodging 'controlled' through selection of clones and careful control of irrigation (Appendix 10)*

The fourth lodging experiment was highly successful in that lodging was 'controlled' through selection of clones for lodging proneness from a segregating population and through careful control of irrigation. These treatments resulted in the desired combination of fully lodged prone cultivars, erect prone cultivates and erect non-prone cultivars at the end of the wet season. We could then observe growth in many yield components from April to August while all plots were irrigated equally.

27. *Lodging did not affect yield components*

Cane yield (fresh mass) increased about 10% from April to August 2008 period regardless of clone or irrigation treatment. Dry matter content of stalks increased markedly over this period resulting in large increases in total and stalk dry biomass. However the increase was not linked to degree of lodging

whether this was assessed at the beginning or end of the April to August growth measurement period. Contrary to expectations lodging due to either genotype or irrigation treatment had no measurable effect on any yield component in this experiment. This is surprising particularly for MQ77-340 which was fully lodged by 1 April and yet continued to accumulate biomass and sucrose at about the same rate as the other clones.

28. Hypothesis that irrigation favours erect clones is not supported

The fourth lodging experiment did not support the hypothesis that irrigation management favours certain clones in selection trials more than others. It did not indicate that clones prone to lodging will be selected preferentially when irrigation is used sparingly. However the experiment did indicate that erect but retarded crops can 'catch up' with more advanced but lodged crops after the wet season but this did not favour clones that were more prone to lodging.

29. Lodging can be delayed, water saved and yield maintained by careful irrigation management

The loss in sucrose yield due to withholding irrigation was 29% in April but was not significant (9%) in August when the crop was harvested. This slight (if any) yield advantage for the liberal irrigation regime came at the cost of an additional 558 mm (5.6 ML/ha) irrigation. This experiment proved that at least in some soils, Burdekin growers could reduce irrigation amounts considerably without significant yield loss.

30. WaterSense can be used to delay lodging and save water

The irrigation treatments applied in the fourth lodging experiment were based on the WaterSense model and are therefore repeatable. However a trickle system would be required to apply the limited but frequent irrigations as in this experiment. No doubt an effective regime could be worked out for a furrow irrigation system.

31. WaterSense could help growers to irrigate for maximum CCS – Appendix 11

The first step to saving water in full irrigation schemes is to irrigate only as much as required to replace water actually used by the crop. This could be achieved by using a water budgeting tool like WaterSense which has been shown to accurately determine the amount of water used by the crop (Webb *et al.* 2006). WaterSense indicates when and how much to apply to replenish water stored in the soil (Fig. 10a). WaterSense can also help with the next phase in water savings by indicating how to exploit water stored deep in the profile. The model indicates when biomass and sucrose accumulation is likely to be reduced by water stress and irrigation can then be scheduled to avoid such stress (Fig. 10b). Deficit irrigation of this nature could save water as well as increase CCS (Appendix 11).

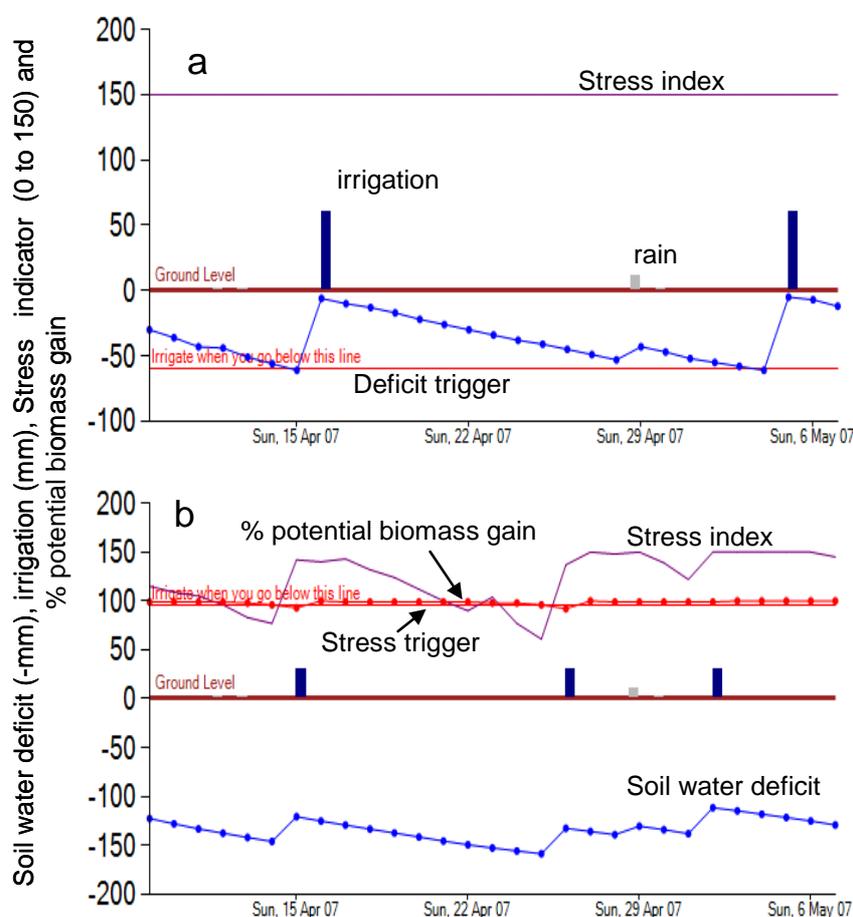


Fig. 10. Graphical displays from WaterSense indicating a) an efficient irrigation schedule replacing 60 mm each time this amount is removed by the crop and b) a more efficient deficit schedule replacing a smaller amount (30 mm) and maintaining a large soil water deficit, reducing leaf and stalk elongation without reducing biomass and sucrose accumulation. The water stress indicator indicates no stress when = 150; indicates limited leaf growth when 100-150; and below 100 indicates reduced photosynthesis and reduced biomass and sucrose accumulation

Evaluation

32. Sugarcane science is starting to link up over all levels of organisation - Appendix 12

The work reviewed and presented in the special issue of Field Crops Research by Casu *et al.* (2005), Watt *et al.* (2005), Rae *et al.* (2005) and Singels *et al.* (2005b) presents an opportunity to link modelling of whole crop behaviour with gene expression and cellular studies and thus span the range of organisational levels from molecule to crop. Experiments designed to provide coefficients for the partitioning model of Singels and Bezuidenhout (2002) could also provide tissue for micro-array analysis, so that genes responsive to up- and down regulation by a range of well defined experimental conditions (water, radiation, temperature) can be identified. This could provide a clearer picture of how

gene expression for sucrose accumulation is regulated by developmental and environmental conditions than has been achieved so far with the limited range of developmental stages considered by Casu *et al.* (2005) and Watt *et al.* (2005). Molecular biologists, plant and crop physiologists, breeders and modellers all need to be involved in this type of research which is a step towards the holistic, top-down/bottom-up approach envisioned by Moore (2005).

33. Physiological knowledge presently contributes very little to trait selection – Appendix 13

The BSES-CSIRO Sugarcane Variety Improvement Program generally selects for cane yield and CCS, disease resistance and to a lesser extent some other traits of economic importance. The decision to select for these traits is primarily made by the Breeding Team. Physiological knowledge presently contributes very little to decisions made regarding trait selection, largely due to the lack of physiological knowledge on sugarcane. Although there is little or no use of physiological models, in particular APSIM, in the BSES-CSIRO breeding program at present, there is a clear intention to increase their use in the future. Breeders thought that the biggest genetic gains in sucrose accumulation would be achievable through improved yields of ratoon crops and improved early-season CCS. Breeders considered the impact of project CSE014 on the BSES-CSIRO Sugarcane Variety Program to be at least moderate.

34. Project is evaluated progressively in workshops – see Appendix 14

The workshop on 16 October 07 acknowledged that considerable progress had been made toward understanding, documenting and modelling source – sink control of sucrose accumulation but that there was nothing yet that could be crystallized out for use in breeding or selection. The breeders present agreed that more knowledge about sucrose physiology was useful even if not directly for selection procedures. A firm suggestion was made that rather than looking for traits to select for we should be looking for those to discard which may be easier and more useful in the early stages of selection. The results of CSE014 experiments highlighted the role of stalk population in sucrose accumulation and there was substantial debate over the role of plant anatomy including stalk population, diameter and length in both sucrose and lodging attributes. Breeders would value any indication of anatomical traits that confer low CCS or lodging tendencies in sugarcane clones so that clones with these traits can be discarded early in the selection process.

A firm recommendation from the steering panel and other expert breeders and physiologists was that we need to consider many more clones than the four that have been used thus far. A suggestion was made that we consider high and low CCS categories from extremes of segregating populations as before but increase the numbers in each category to 30 or more. Interest in the individual clones would then be sacrificed for interest in the general nature of high and low CCS categories. This approach aligns with the molecular work where CCS is related to a large number of markers in a large number of clones.

35. Interim assessment of physiological basis of variety improvement

The model for whole plant sucrose accumulation (Inman-Bamber *et al.* 2009) and the recent model of sucrose accumulation by internodes of sugarcane developed by Dr Singels are bringing us closer to identifying sub-traits for sucrose accumulation in sugarcane. Evidence from this project presented by Inman-Bamber *et al.* (2009) indicated that photosynthesis in high- and low sucrose clones is similar and that there is no limitation in the storage tissue of low sucrose clones for high sucrose content. Differences between clones were found in their responsiveness to water stress which reduced expansive growth more than photosynthesis thus altering the source-sink balance to a lesser extent in low than in high sucrose clones. Differences in high- and low sucrose clones were also found in the morphology of plants such that high sucrose clones produced fewer stalks than low sucrose clones. Low sucrose clones allocated more dry matter to leaves than high sucrose clones possibly to maintain a

minimum leaf area for each stalk. It is conceivable that differences between high- and low- sucrose clones may be explained by source-sink attributes as presented by Inman-Bamber *et al.* (2009) and that the biochemistry of tissue which is up- or down regulated for parts of the sucrose accumulation pathway may be an effect rather than a cause of the difference. These are novel observations from a whole plant perspective on sucrose accumulation in sugarcane.

Dr Singels' model identified other sub-traits that distinguish high- from low- sucrose clones albeit supported by only four clones. Leaves of low sucrose types emerged slower than those of high sucrose types but tillers emerged more rapidly in low sucrose types. Biomass accumulation was lower for low sucrose types, particularly for KQ97-2599, than for high sucrose types. The leaf partitioning coefficient was greater for low than for high sucrose types. Interestingly the maximum storage capacity was not distinctly higher for high sucrose types.

When this model is improved and validated using other datasets we will be able to assess *ideo-types* in silico.

36. Opportunities for international collaboration

The Institute for Plant Biotechnology (IPB) in Stellenbosch, S. Africa, have developed a modified version of NCo310 which is down regulated for glycolysis and PFP activity. These plants will go to field trials in April and this provides an ideal opportunity to test Dr Singels internode level model on highly modified plants. Also a new project funded by SRDC (CSE023) on feed back inhibition of photosynthesis of leaf segments and whole plants, complements several SASRI projects on the subject. Dr Riekert van Heerden is an expert in chlorophyll fluorescence which could be important for determining feedback responses in CSE023. It may be possible for Riekert to visit Australia to help with this project.

37. Contribution of project to breeding technology evaluated against baseline

While people in this sugarcane improvement program notably Drs Phil Jackson, Nils Berding, Prakash Lakshmanan and Graham Bonnett have been intimately involved in the project we cannot claim that anything has changed in the breeding program as a result of this work. The project offered a fresh challenge to the molecular biologists to look at the whole plant rather than just the tissue in which sucrose accumulates. The project has taken the argument for a top down and bottom up approach, to those involved in breeding and selection, molecular biology and plant physiology to think about plant improvement and production as a whole system. Thus those working on feedback inhibition by sucrose were encouraged at the SASRI workshop in March 09 to think of other reasons why sucrose accumulation declined after removing leaves, before going to the expense of identifying processes and genes involved in feed back inhibition. Those identifying genes involved in up or down regulation of sucrose accumulation were also challenged to look at the whole plant to ensure that they are working with the causes of sucrose accumulation rather than the effects of some other cause as suggested by Inman-Bamber *et al.* (2009). The internode sucrose accumulation model built by Dr Singels was offered as a framework on which to build our knowledge of the sucrose accumulation process. The model explains sucrose accumulation by maintaining the mass balance of carbohydrate generation (photosynthesis) and distribution to the various plant components and to each internode. We believe that this framework provides the best chance for a comprehensive understanding of the complex interactions between the climatic and cell environments in the production of sucrose. The impact on the breeding program will then occur through the capability to evaluate sub-traits of sucrose accumulation in the context of climate variability over space and time, in the Australian sugar industry.

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 of the results as they are analysed and interpreted.

ENVIRONMENTAL AND SOCIAL IMPACTS

This project is of long-term strategic significance (horizon 3) and has no negative environmental or social impacts. The most immediate positive impact is the development of technology to limit irrigation in order to enhance CCS and reduce lodging. While the aim of this technology is to improve the economics of cane growing the environmental benefits for using less water are substantial. Reduced irrigation means reduced drainage and runoff as well as conservation of scarce water resources. If drainage and runoff are reduced then nutrients and pesticides in water leaving sugarcane fields will also be reduced.

EXPECTED OUTCOMES

When this project commenced, sound physiological explanations for differences in sucrose accumulation and sucrose content among sugarcane clones and cultivars did not exist despite the considerable effort to identify genes, enzymes, and biochemical pathways that might differ among high- and low-sucrose genotypes. This project endeavoured to restore some balance in the approaches worldwide for breaking through the apparent barrier to improved sucrose content now limiting progress in sugarcane breeding. The project raised important questions about research at cellular and sub-cellular levels where most of the effort has been directed for improving sucrose content. We showed that clones deliberately chosen for low sucrose content could be coaxed to store high concentrations of sucrose in some internodes so the biochemistry and cytology of the storage tissue in isolation may not reveal much about limits to sucrose storage.

This project and its predecessor (CSE006) gave rise to a large number (20) of journal articles which document our current knowledge of the sugarcane plant and its complex relationship to the environment. While some of these papers have been published only recently those published in the *Field Crops Research* journal in 2005, have been cited 131 times to date with an impact factor of 7.2, more than 3 times higher than for the journal itself. Sugarcane physiology research, while limited in Australia compared other countries, has attracted considerable attention, thus allowing us to freely exchange knowledge generated in these other industries. For example chief investigators Geoff Bamber and Graham Bonnett were invited to select symposia in Brazil and South Africa in 2008 and 2009. Geoff was one of three international speakers (including Dr Paul Moore) to address a 3 –day symposium in Brazil in May 2008. Many contacts were made at this symposium leading to the application of two students to come to Australia to work with Geoff. Graham was invited to speak on physiology at a workshop on Sugarcane Improvement in Sao Paulo organised by the BIOEN group who are looking at the development of energy crops, and systems for exploiting them. The workshop was in March 2009 and work generated in the project was discussed. Our reputation also led to a six month visit to Townsville by Dr Singels (SASRI) who developed the internode level sucrose accumulation model, a significant output from this project.

Benefits of this strategic project (horizon 3) are expected in the long term and the pathway to these benefits has been defined in terms of the modelling framework required to link research across different levels of organisation of the plant. Australia has taken the lead in this type of gene to phenotype modelling in other crops where the vision is to design crops to match variable economic and environmental requirements by understanding the role of genes or markers in relation to individual traits. These traits can interact with the environment in complex ways such that they confer benefits in some situations (regions or years) and disadvantages in others. Gene to phenotype models are being developed to identify both the environment in which selections for improved crops are made and the genes, markers and traits required to exploit a

given environment. The position with sugarcane at the outset of this project was a dearth of knowledge about sub-traits that make for differences amongst genotypes in regard to sucrose accumulation, the most important process in the sugar industry. We have now made a good start in catching up with other cropping systems in linking genetics and the environment (including management) through crop and plant physiology, having begun to fill the gap in knowledge clearly identified at the 2003 physiology workshop. Interestingly there is a large amount of overlap in the optimum 'design' of grain crops and sugarcane, with a focus on redirecting photo-assimilate to the commercially valuable plant component. Techniques for monitoring and modifying such redirection also overlap with a focus on photosynthesis, tillering and plant extension rate in both grain and sugarcane crops.

Despite the strategic nature of the project some results can be applied immediately and we demonstrated in the report how WaterSense can be used to control elongation growth, lodging and CCS in farming practice, at least to some extent. Benefits have WaterSense have been demonstrated in other projects (CSE007 and BSS297) as well. This report provided examples of how irrigation can be managed carefully to save water and at the same time maintain or even increase sucrose yields. Some growers are recognising these benefits for themselves but demonstration sites may be useful for convincing others that irrigation can be managed more scientifically for these benefits.

FUTURE RESEARCH NEEDS

Several papers that were initiated during the project need to be completed. This without question is the best first step in capitalising on the investment in this project. These papers provide a permanent record of the nature of the research undertaken, accessible to the scientific community, to local industry and to SRDC. Papers like this ensure that the data produced, at great cost, is used to the best advantage with a high degree of rigour. All aspects of the work particularly the conclusions are open to scholarly review and criticism in this way. After all work from this project is published SRDC will be able to assess any future project proposal in the light of what is known already. This will ensure that future work increases our knowledge base rather than simply complementing it. In addition to the standard practice of publishing, the knowledge generated in this project needs to be embedded in working models of the sugarcane cropping system. We made a start in this project in developing the internode sucrose accumulation model. This now needs to be linked or embedded in a whole crop model like APSIM or Canegro.

RECOMMENDATIONS

Two recent projects on sugarcane physiology have started with SRDC funds, one on high photosynthesis (CSE023) and another on elevated CO₂. These projects will add to knowledge of source-sink relationships that appear to be fundamental in the allocation of photo-assimilate to sucrose or fibre. We recommend that SRDC continue funding this type of research, over the long term and help facilitate continuity in the efforts to link research at molecular to mill levels particularly with help of dedicated computer models. Linking genes with phenotypes is an international endeavour in any cropping system so we recommend that SRDC help retain expertise in the sugar industry capable of contributing and benefitting from such an international effort. From the literature it appears that the Brazilians are intensifying plant physiology research on elevated CO₂ and water relations. There is a renewed interest in Brazil in irrigation as the industry expands into drier areas and poorer soils. We have already mentioned the interest in feedback inhibition of sucrose on photosynthesis by South African scientists and we need to interact with SASRI in this regard possibly by hosting visiting scientists or visiting SASRI ourselves.

The International Consortium on Sugarcane Modelling (ICSM) has recently called for expressions of interest in research to understand the GxE interaction across world sugar industries. This is an important initiative.

Why do locally bred and selected clones perform well only in the country of origin when climatic conditions in other countries appear to be similar? This project could help us get closer to the basic physiological process that make for differences in genotypes thus allowing us to link genes, traits and phenotypes in our models. It is important that the Australian Sugar Industry join with from this international effort. This may require some out of cycle funding.

PUBLICATIONS FROM THE PROJECT

Journal articles

1. Inman-Bamber NG (2008) Economic impact of water stress on sugar production in Australia. *Sugar Cane International*. 25, 7-11. (Appendix 17)
2. Inman-Bamber, NG, Bonnett, GD, Spillman MF, Hewitt ML, Jackson J (2008) Increasing sucrose accumulation in sugarcane by manipulating leaf extension and photosynthesis with irrigation . *Australian Journal of Agricultural Research*. 59, 13 - 26. Feature article for this issue.
3. Inman-Bamber, NG, Bonnett GD, Thorburn PJ, Garside A, Berding N, Attard, SJ (2008) Sugestões para o melhoramento da agricultura e da pesquisa baseadas na fisiologia da cana-de-açúcar. *International Sugar Journal*. 110, 583-588. (Appendix 16)
4. Inman-Bamber NG, Bonnett GD, Spillman MF, Hewitt ML, Jingsheng Xu (2009) Source-sink differences in genotypes and water regimes influencing sucrose accumulation in sugarcane stalks. *Crop and Pasture Science*. 60, 316–327.
5. Inman-Bamber NG, Bonnett G D, Spillman MF, Hewitt ML, Glassop D (2009). Source–sink differences in genotypes, temperature regimes and thinning treatments influencing sucrose accumulation in sugarcane stalks. *Crop and Pasture Science* (in review)

Refereed conference papers

6. Attard SJ, Inman-Bamber NG and Hesp C (2009). Evaluating alternative irrigation in the lower burdekin in terms of the water balance. *Proceedings Australian Society Sugar Cane Technologists*. 31, 230-239.
7. Inman-Bamber NG (2008). Whole plant and crop perspective of the physiology of sucrose accumulation in sugarcane 13-15 May 2008, Botucatu, Brazil.
8. Inman-Bamber NG, Attard SJ (2008) Water savings and water accounting in irrigated sugarcane. *Proceedings Australian Society Sugar Cane Technologists*, 30, 251-259.
9. Inman-Bamber NG, Bonnett GD, Thorburn PJ, Garside A, Berding N and Attard SJ (2008) Pointers for better farming and research from sugarcane physiology. *Proceedings Australian Society Sugar Cane Technologists*, 30: 231-240.
10. Inman-Bamber NG, Attard SJ, Haines MG, and Linedale AI (2008) Deficit irrigation in sugarcane using the WaterSense scheduling tool. *Irrigation Australia Conference, Melbourne 20-22 May 2008*.

11. Singels A and Inman-Bamber NG (2009) Phytomer level source-sink model of biomass production and partitioning in sugarcane. Proceedings of the South African Sugar Technologists Association, 83 (in press).

Popular articles

12. Attard, Steve and Inman-Bamber, Geoff (2007). Factors affecting the optimal growth of your crop. Australian CANEGROWER. March 2007, pp 12-13.
13. Inman-Bamber, Geoff and Attard, Steve (2007). How cane makes sugar while the sun shines. Australian CANEGROWER. February 2007, pp 18-19.
14. Inman-Bamber, Geoff and Attard, Steve (2006). Sunlight a critical factor on cane yield and CCS. Australian CANEGROWER. December 2006, p17.
15. Inman-Bamber, Geoff, Attard, Steve and Berding, Nils (2006). The link between lodging and reduced sugar. Australian CANEGROWER. October 2006 p20.
16. Inman-Bamber, Geoff and Attard, Steve (2006). Clever irrigation can help roots go deeper. Australian CANEGROWER. September 2006, p12.
17. Inman-Bamber, Geoff, Attard, Steve and Garside, Alan (2006). The myth surrounding stalk density and yield. Australian CANEGROWER. August 2006 pp 16-17.
18. Inman-Bamber, Geoff and Attard, Steve (2006). Why a July ratoon out-yields an October ratoon. Australian CANEGROWER. July 2006, p18.

Radio interview

ABC Far North (Cairns), Rural Report, 12/06/2007 06:15AM Compere: Adam Stephen. Stephen speaks with Geoff Inman-Bamber, Principal research scientist, CSIRO, about studies into the costs and impact of water stress on the sugar industry, which he puts at \$250m a year.

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LIST OF APPENDICES

1. Increasing sucrose accumulation in sugarcane by manipulating leaf extension and photosynthesis with irrigation
2. Source–sink differences in genotypes and water regimes influencing sucrose accumulation in sugarcane stalks
3. Source–sink differences in genotypes, temperature regimes and thinning treatments influencing sucrose accumulation in sugarcane stalks
4. Physiological and morphological differences between sugarcane clones with different sucrose contents
5. Phytomer level source-sink model of biomass production and partitioning in sugarcane
6. Lodging pilot trial results to date and suggested changes to the experiment
7. Report on Lodging with irrigation pilot experiment, first ratoon (06/07)
8. Report on Clone x Lodging with irrigation experiment, plant crop (06/07)
9. Choice of clones for the lodging experiment
10. Report on Clone x Lodging with irrigation experiment, ratoon crop (07/08)
11. Water savings and water accounting in irrigated sugarcane
12. Directions for R&D and cane growing from an international review on sugarcane physiology
13. Knowledge, Attitudes, Skills and Aspirations of key members of the BSES-CSIRO-CSR Sugarcane Variety Improvement Program
14. Progressive evaluation project CSE014
15. First Brazilian Symposium on Sugarcane Ripening
16. Sugestões para o melhoramento da agricultura e da pesquisa baseadas na fisiologia da cana-de-açúcar. *International Sugar Journal*, 110, 583-588.
17. Economic impact of water stress on sugar production in Australia. *Sugar Cane International*. 25, 7-11.
18. Articles in the Australian Canegrower Magazine

Appendix 4
Physiological and morphological differences between sugarcane clones with different sucrose contents

NG Inman-Bamber, GD Bonnett, D Glassop, MF Spillman, M Hewitt.

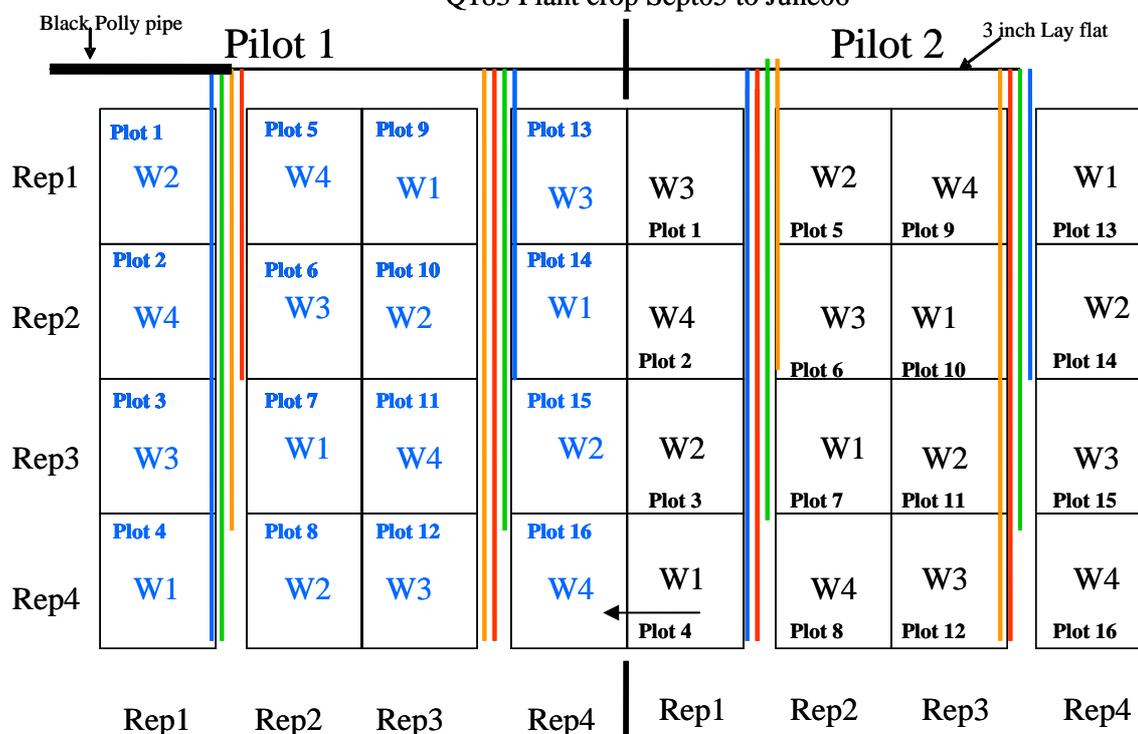
Early draft paper for Crop and Pasture Science (2010?)

Appendix 6
Lodging pilot trial results to date and suggested changes to the experiment.
G Bamber 27/6/06

A trial was planted at Kalamia in September last year to see how we can reduce lodging while achieving high yields. The concept was to gain control over plant height through irrigation, reducing plant height before the wet season to reduce lodging and then to allow both lodged and erect treatments to grow with irrigation to see to what extent the shorter and more erect treatments can catch up or even overtake the lodged ones. This is in preparation of a lodging by clone experiment where the clones are selected for susceptibility or resistance to lodging and where the object is to determine the role of management (environment) on the expression of the lodging trait.

Layout for growth control pilot trial in Field 62-1 Kalamia

Q183 Plant crop Sept05 to June06



Treatments

The treatments were designed to encourage or discourage lodging without causing excessive water stress. Auxanometers were installed to monitor plant extension rate as a means of assessing the degree of water stressed sensed by the plants. Irrigation treatments were based on frequency rather than amount, the amount being limited to what could be applied before water started to pond and then run off the plots.

Irrigations was applied to plots in the following manner after 7 February 06. The last irrigation (furrow) was applied to all plots on 20 December 05.

W1 = frequent irrigation (every week day)

W2 = less frequent (twice a week)

W3 = not irrigated

W4 = not irrigated

Results to date

1) Daily plant extension rate (PER)

PER was greater in W1 than in the other treatments during February but then dropped below PER of the other treatments during March (Fig. 1). During February PER was mostly greater in W2 than in W3 as would be expected but PER in W4 was greater than in W3 even though W4 was also not irrigated after 20/12/06. PER differed little between W2, W3 and W4 during March. The small differences in PER indicate that rainfall during this period was sufficient to minimize differences in biomass yield between treatments. We would only expect a difference in cane yield or biomass if PER was reduced by more than 50 %.

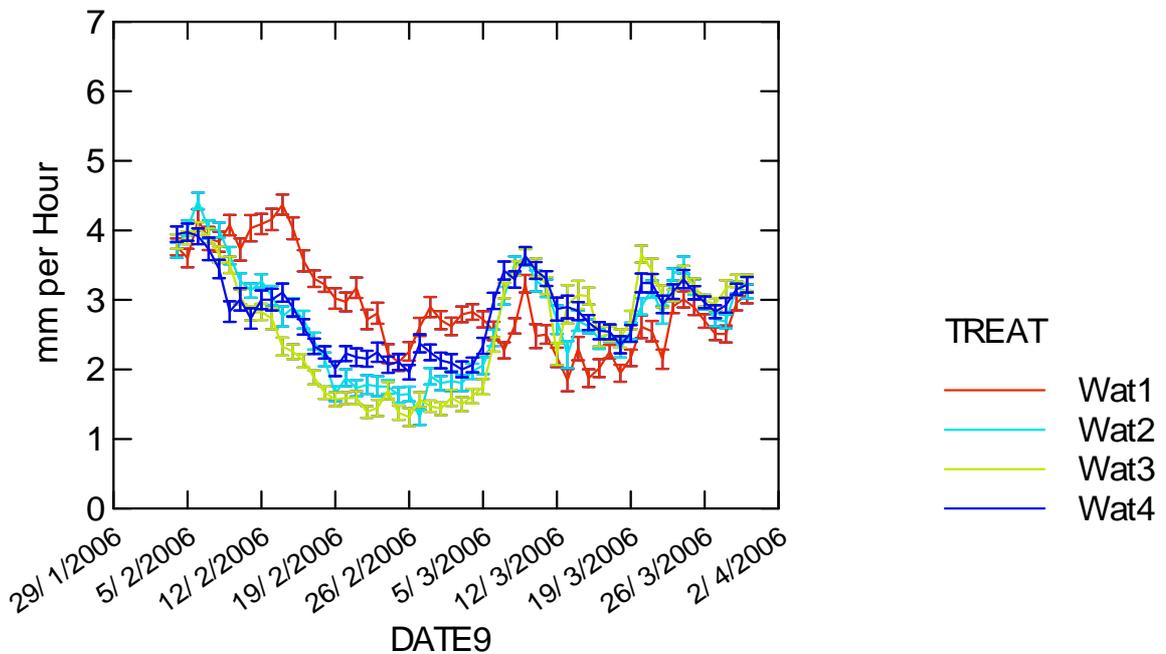


Fig 1. Daily mean plant rate (mm per hour) for four irrigation treatments. Bars indicate 2X standard error of the mean.

2) Lodging

Lodging was rated twice by different people and the results were statistically significant ($p < 0.05$) on both occasions with lodging in W1 greater than in the other treatments (Fig 2).

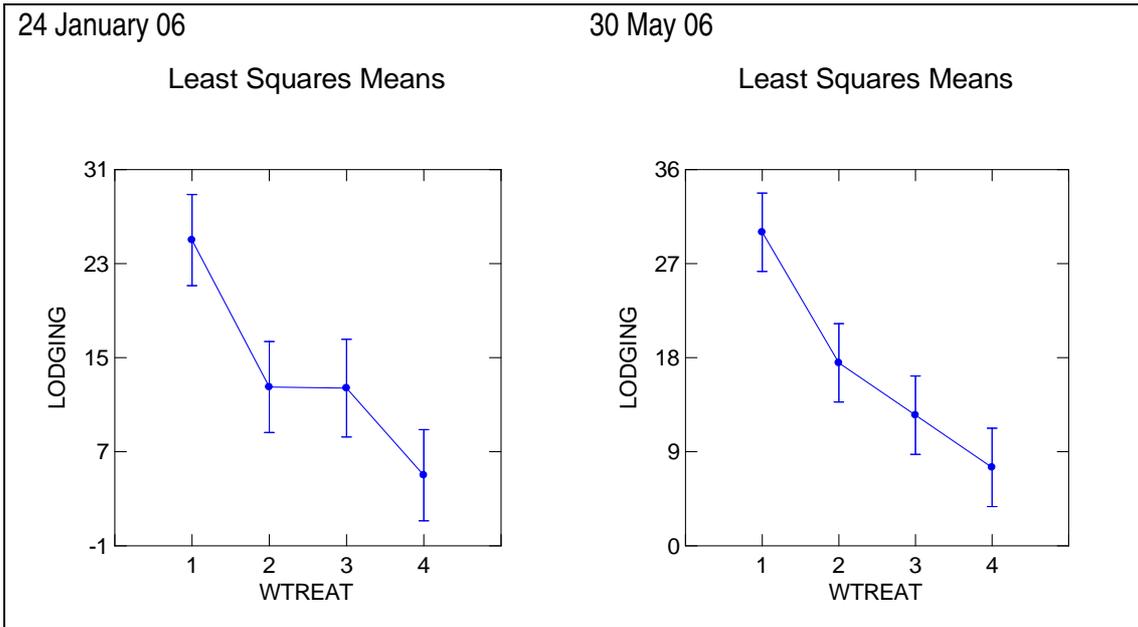


Fig 2. Mean lodging angle (0 = all stalks vertical, 90 = all stalks horizontal) rated on 24 January and 30 May 2006 for four irrigation treatments (note change in Y scale).

An attempt was made to rate lodging on 15 June 06 but most of the trial was lodged regardless of previous irrigation treatment (see photo). While some control of lodging was achieved we could not avoid the extensive lodging that has now occurred. It will not be possible determine growth rates (biomass and sucrose accumulation) after the wet season in erect and lodged cane as was hoped.



3) Suggested changes to plan.

We suggest harvesting the trial as soon as possible so that irrigation treatments can be applied as early as September to encourage a range of lodging risks (stalk height and wet soil) by late December. This will give us the best chance of getting experience with control of lodging by irrigation, for the experiment on clones with different lodging tendencies (critical trial). It is a pity that we will not have had this experience beforehand but the repeated pilot trial will allow us to consider four irrigation treatments whereas the experiment with clones will only accommodate two irrigation treatments. The pilot and critical trials will run concurrently and two of the treatments of the pilot trial will be used in the critical trial. We suggest using the wettest treatment (irrigation every week day for example) and the driest but one, in the critical trial. The pilot trial will give us an idea of what might have happened had we used one of the other treatments (the wettest would always be used) in the critical trial and if need be the critical trial can be ratooned and repeated with a better selection of the sub-optimal irrigation treatment which is designed to hold back the crop to reduce lodging and to enhance growth after the wet season. This is expected to benefit clones that lodge easily more than those that don't.

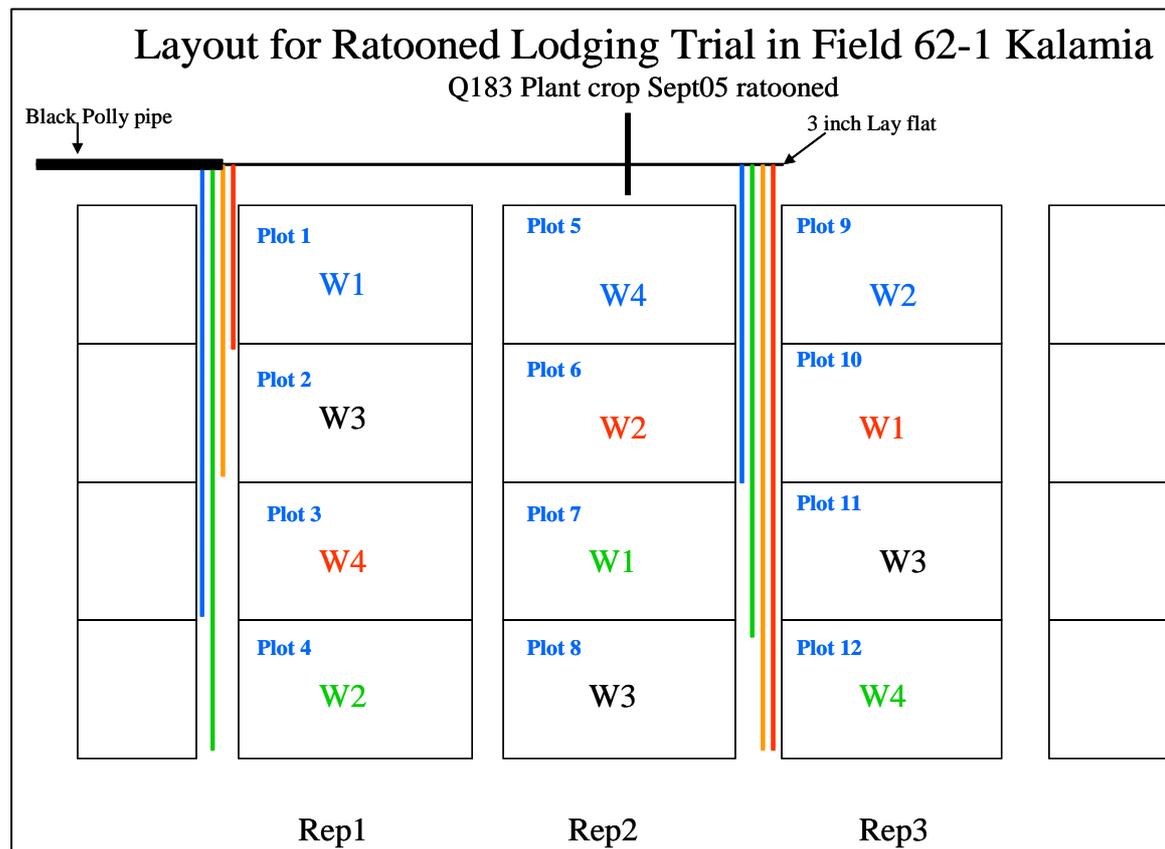
Appendix 7

Report on Lodging with irrigation pilot experiment, first ratoon (06/07)

CSR Kalamia Estate

NG Inman-Bamber, October 2007

A trial was planted at Kalamia in September 2005 to see how we can reduce lodging while achieving high yields. The concept was to gain control over plant height through irrigation, reducing plant height before the wet season to reduce lodging and then to allow both lodged and erect treatments to grow with irrigation to see to what extent the shorter and more erect treatments can catch up or even overtake the lodged ones. The wet season arrived before being able to delay stalk elongation through reduced irrigation and it was decided to harvest the trial as soon as possible in 2006 so that irrigation treatments can be applied as early as September to encourage a range of lodging risks (stalk height and wet soil) by late December. The experimental block was harvested on 26/7/06



Irrigation treatments

The treatments were designed to encourage or discourage lodging without causing excessive water stress. Auxanometers were installed to monitor plant extension rate as a means of assessing the degree of water stressed sensed by the plants. Irrigation treatments were based

on frequency rather than amount, the amount being limited to what could be applied before water started to pond and then run off the plots.

The following decisions to implement and change irrigation treatments were based on plant extension measurements and the objective of reducing plant height by about 50% before the wet season. We aimed to achieve the tallest possible plants and earliest lodging in a 'W1' treatment by frequent irrigation. The greatest reduction in growth and longest delay in lodging was planned for a W4 treatment while W2 and W3 treatments were intended to be intermediate.

10/10/06 All irrigations were 3 hours each
 W1 irrigated every week day,
 W2 irrigated twice a week (Mondays, Thursdays)
 W3 and W4 not irrigated at all.

8/11/06
 W1. Irrigation every week day (as before)
 W2. Irrigation twice a week (as before)
 W3. Irrigation twice a week (same as W2), since difference has already been achieved
 W4. Irrigation once a week

7/12/06
 W2 was getting too far behind and new regime was implemented:

	Mon	Tue	Wed	Thu	Fri
W1	4 hours	3 hours	3 hours	3 hours	3 hours
W2	4 hours			3 hours	
W3		3 hours			3 hours
W4			3 hours		

12/1/07
 Decided to water for 4 hr on Friday to help growth over weekend in W1 plots

	Mon	Tue	Wed	Thu	Fri
W1	4 hours	3 hours	3 hours	3 hours	4 hours
W2	4 hours			3 hours	
W3		3 hours			4 hours
W4			3 hours		

20/2/07
 The desired difference in crop height and yield had been achieved and it was decided to water all treatments fully, for 8 hours on Tuesdays and Fridays.

Plant and stalk extension and climate

Daily mean plant extension rate (PER) in the W1 treatment exceeded 4 mm h^{-1} in early to mid November when the crop was about 6 months old and minimum temperature exceeded 20°C (Fig 1). PER for the other treatments was considerably lower and surprising low for W2 which was irrigated far often then W3 and W4. For all treatments that were not being irrigated every week day PER responded to rainfall in early December and early January indicating that these treatments were being retarded as intended by water stress. PER for W1 decreased after the December rain and this could indicate some water-logging or reduced growth due to reduced temperature at this time. PER was similar in all treatments after the rains in January and measurements were stopped for this reason and because stalks were now beyond safe reach of the auxanometers.

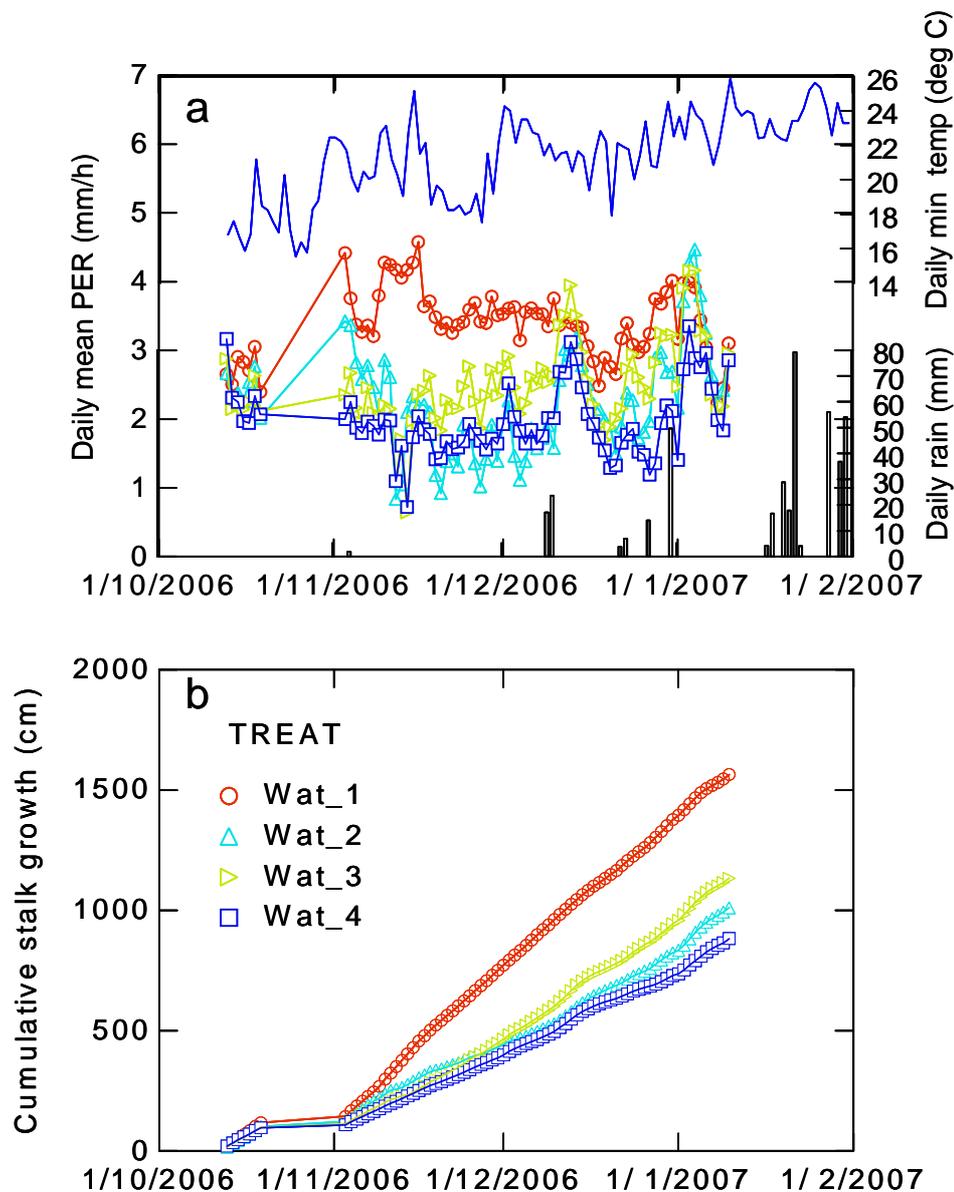


Figure 1 Daily mean PER, minimum daily temperature and daily rainfall (a) and cumulative stalk growth (b) assuming stalks contribute 25% to the extension of the whole plant.

W1 stalks connected to the instruments (auxanometers) accumulated about 70 cm more length than W4 stalks over the six week period starting on 1 November (Fig 1b).

PER differed between treatments mainly in the middle of the day when W4 plants in particular were often growing only very slowly due to water stress while W1 plants were still growing rapidly albeit at a somewhat reduced rate compared to evening growth which was often relatively high (Fig 2).

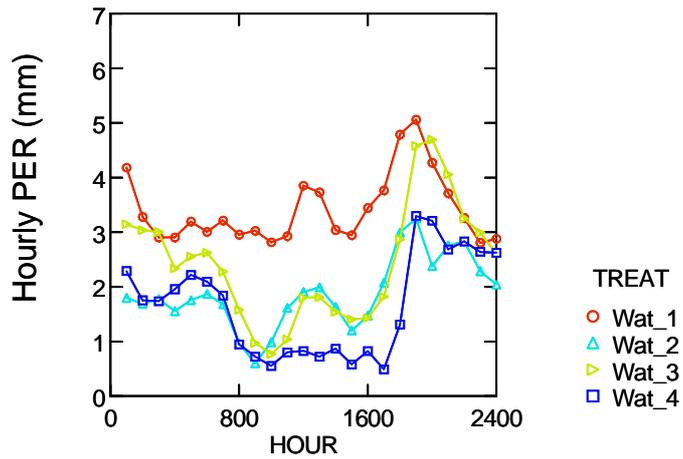


Figure 2 Hourly PER for 23 November 2006

Treatment effect on lodging

Lodging was rated on four occasions and was affected significantly by irrigation treatment in each case (Fig 3a). However by mid April the W1 treatment was only partly lodged despite stalks approaching a height of 4 m (Fig 3b). Strong wind and rain on 8 June caused the whole trial to lodge badly regardless of previous treatment.

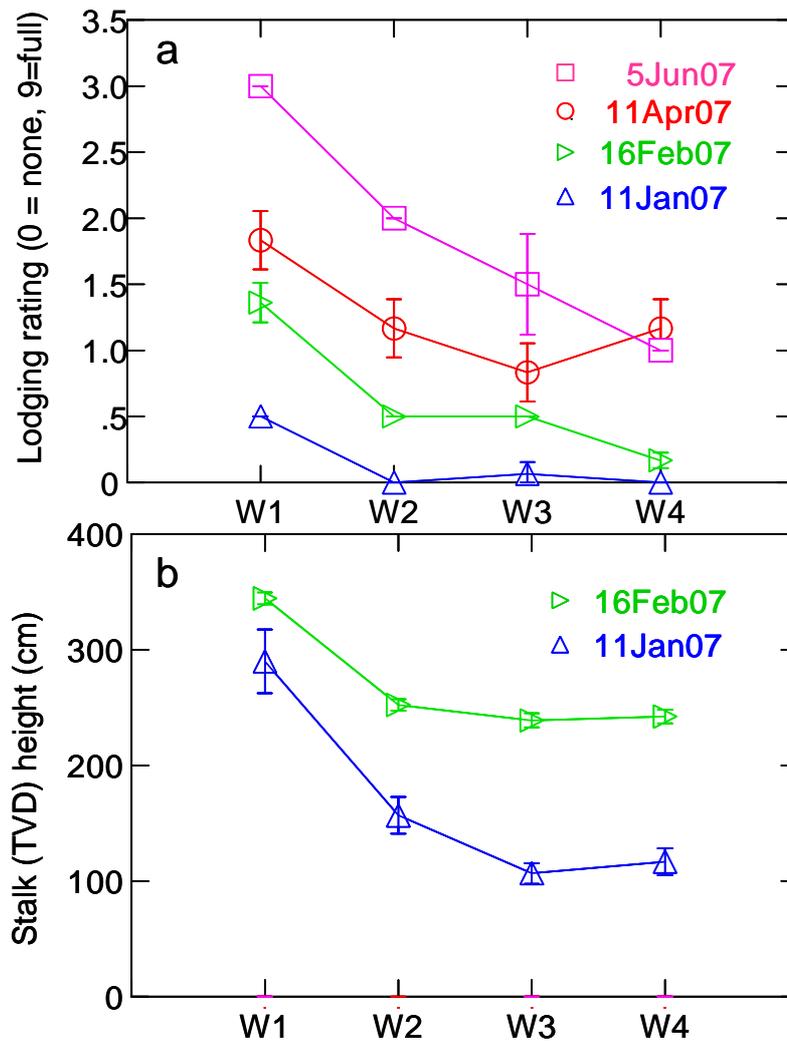
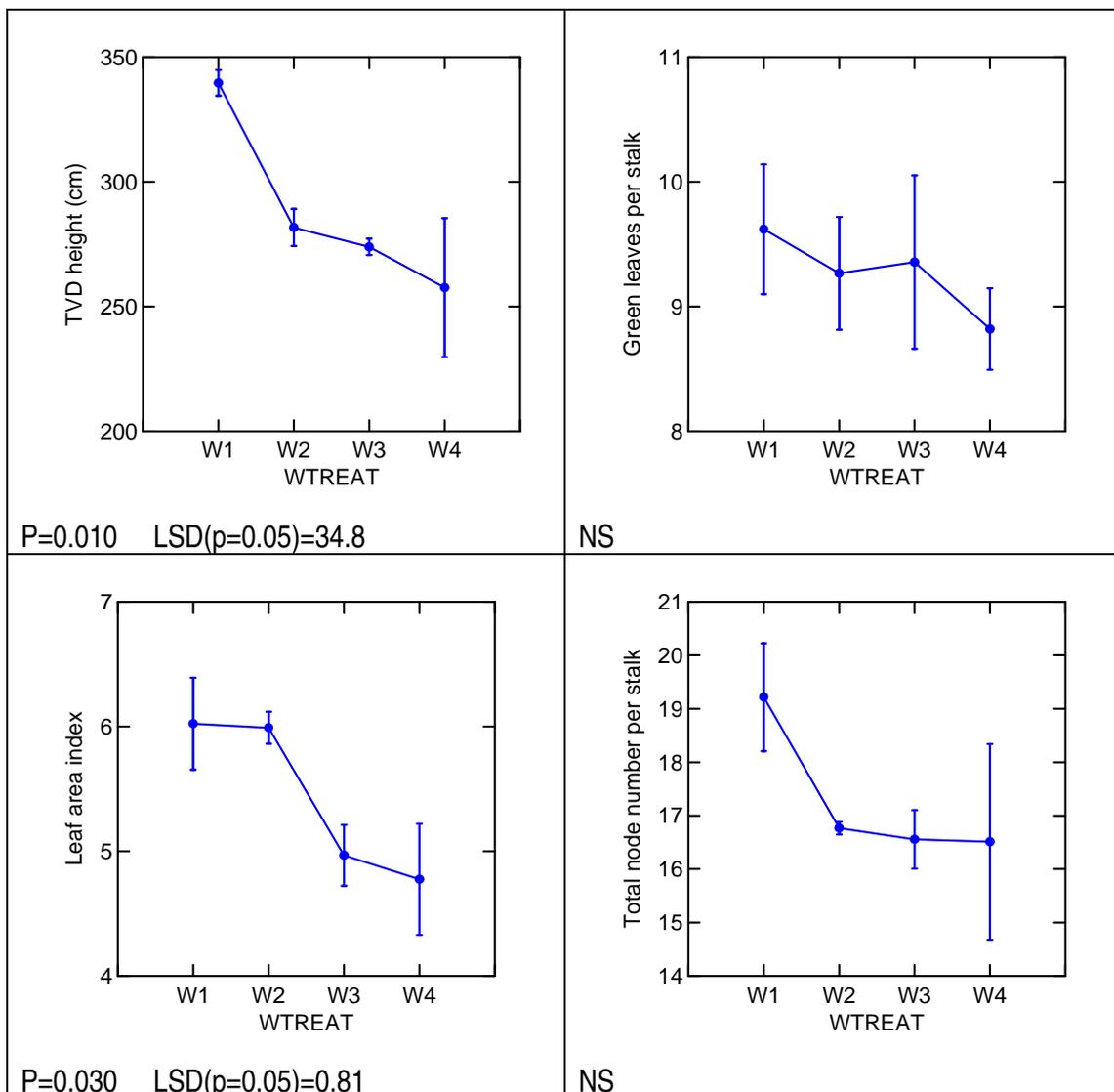


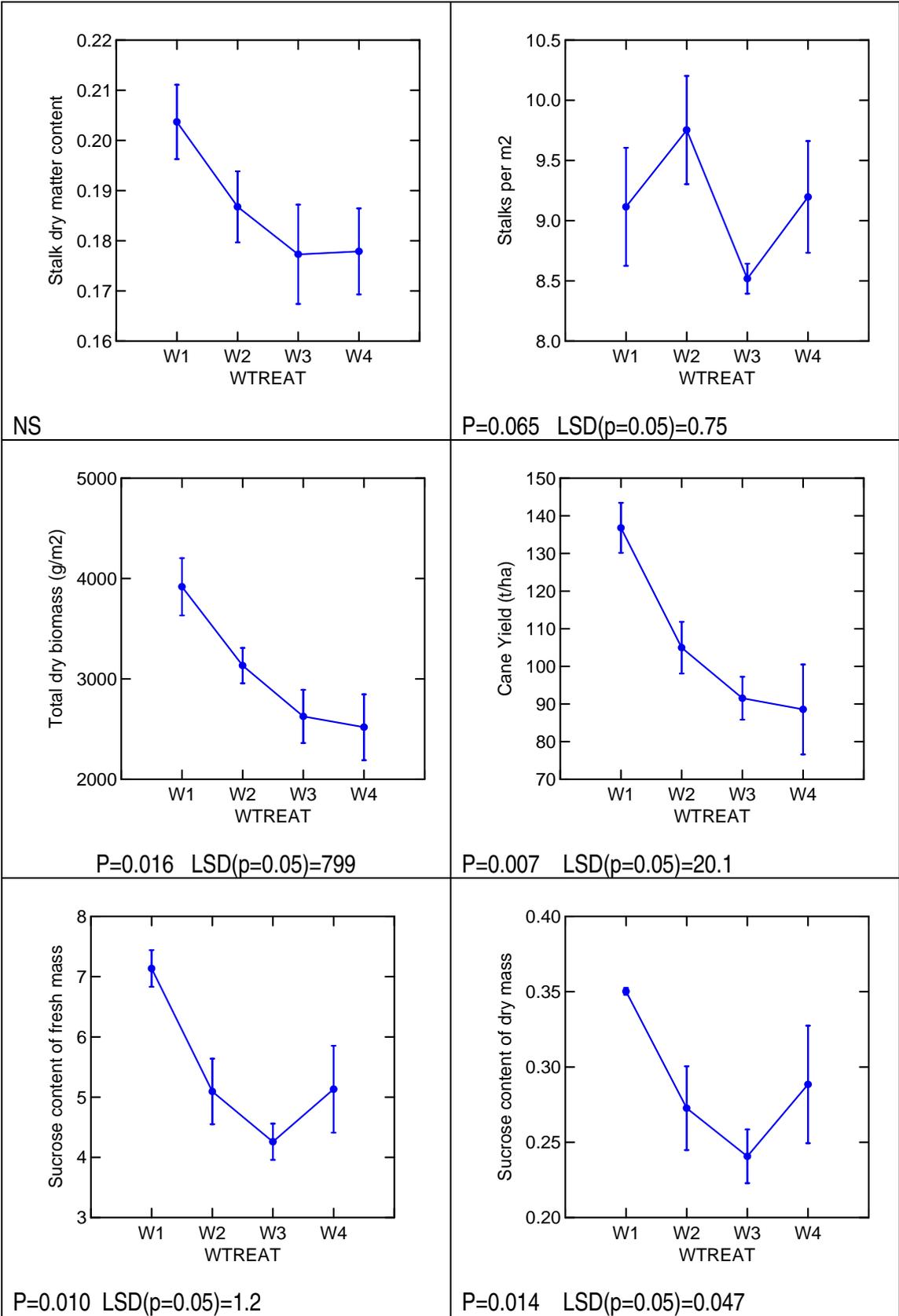
Figure 3. Lodging rating on four occasions (a) and stalk TVD height on two occasions

Plant sampling results (sampled on 7 March 2007)

Irrigation treatment effects were significant for, TVD height, leaf area index, total dry biomass, cane yield, sucrose content of fresh and dry mass and for sucrose yield (Fig 4). Differences between W1 and W2 were large and between W2, W3 and W4 differences were small for all these attributes apart from leaf area index. A comparison of stalk heights on 11 January, 16 February (Fig 3) and 7 March (Fig 4) indicated that the difference between the W1 treatment on the one hand and W2, W3 and W4 treatments on the other was narrowing. The experiment was designed to assess the extent to which more erect plants can catch up with lodged plants and it is possible that the drier and more erect treatments may have caught up with the W1 crop had the trial not lodged badly in June.

The results of sampling in March supported the belief that the more water the better it is for cane and sucrose yield. The W1 crop was approaching 140 t/ha when only 9 months old compared to only 105 t/ha for the W2 treatment.





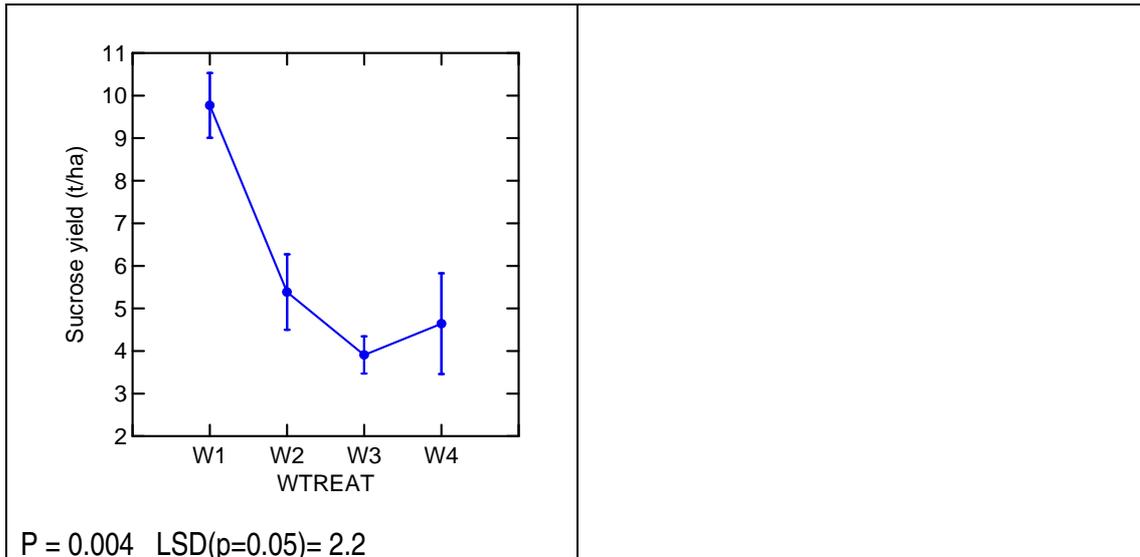


Figure 4. Yields and other attributes determined by sampling on 7 March 2007

Cane yield from bin weights on 29 August 2007

Analysis of Variance

Source	Sum-of-Squares	DF	Mean-Square	F-ratio	P
Reps	1107.3	2	553.6	1.4	0.316
Water treatment	3688.5	3	1229.5	3.1	0.109
Error	2367.0	6	394.5		

Treatment means

W1	t/ha	167
W2	t/ha	129
W3	t/ha	148
W4	t/ha	122

While bin weights may not be very accurate particularly because of the lodged nature of the cane it was evident that the retarded treatments (W2, W3 and W4) did not catch with the well watered treatment which went on to produce 167 t/ha. Treatment differences were not statistically significant but it would not be fair to conclude that treatments had no effect on cane yield only that there is a 10% chance that the differences observed were purely random.

Appendix 8
Report on Clone x Lodging with irrigation experiment, plant crop (06/07)
CSR Kalamia Estate
 NG Inman-Bamber, October 2007

This experiment was designed to assess whether irrigation management favours certain clones in selection trials more than others. It is possible that clones prone to lodging will be selected preferentially when irrigation is used sparingly because lodging is delayed compared to a fully irrigated and more commercial situation. The experiment is also designed to assess the capability for erect but retarded crops to catch up with more advanced but lodged crops after the wet season. Clones that are more prone to lodging should benefit more from the delayed growth pattern.

Treatments

Cultivars

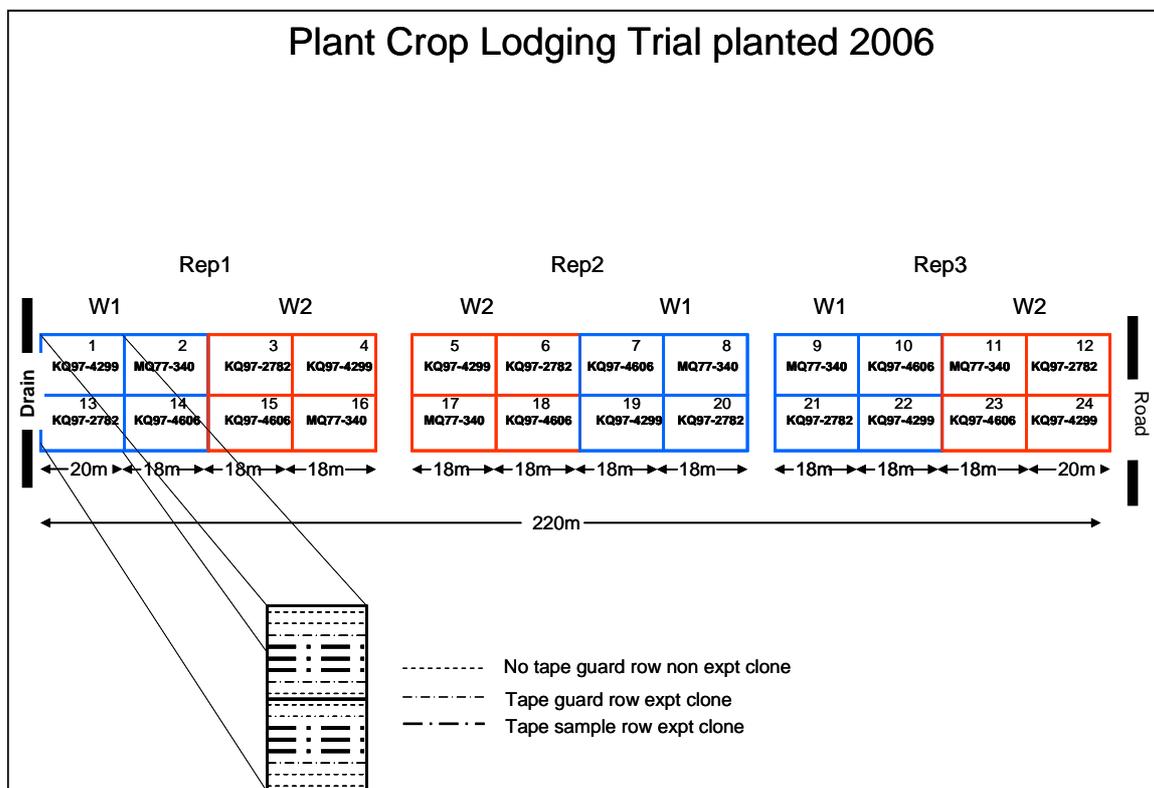
- a. High lodging X 2
- b. Low lodging X 2

Irrigation regimes:

- a. Frequent irrigation to encourage stalk elongation and lodging (W1)
- b. In frequent irrigation to delay stalk elongation and lodging (W2)

Design

The design was a split plot with three replications; whole plots for irrigation treatment and sub-plot for clones.



Choice of clones

Choice of clones for the experiment was based on lodging ratings reported in milestone 4 and on the quality of seed available in the propagation plots. KQ97-4299 and KQ97-4606 ranked third as the most erect clones in at least one of the lodging assessments of the propagation plots and MQ77-340 ranked fourth and KQ97-2782 second as the most lodged of the 34 clones and cultivars.

Planting

10/7/06: Seed was cut from the propagation plots and many buds were swollen on lodging types KQ97-2387 and MQ77-340 which also had long internodes hence fewer buds. Seed material was selected carefully to ensure that no more than two eyes were missing or damaged per 2m stalk.

11/7/06: The trial was machine planted, two setts at time.

13/9/06: When leaves were about 30-40 cm high (5-6 leaves) the three net rows were gapped up by transplanting stools from guard areas particularly for KQ97-2782.

Irrigation

Furrow irrigation was applied several times equally to all plots to achieve establishment.

16/11/06. Trickle tape was installed with two lines of tape per row in the W1 treatment and only one line per row in the W2 treatment. The following irrigation regime was followed.

Treatment	Mon	Tue	Wed	Thu	Fri
W1	4 hours	3 hours	3 hours	3 hours	3 hours
W2	4 hours	3 hours		3 hours	3 hours

7/3/07

All plots looked very wet and W2 was growing faster than W1 (other way around is required) so treatments were change as follows:

	Mon	Tue	Wed	Thu	Fri
W1	3 hours		3 hours		3 hours
W2			3 hours		

Plant extension rate (PER from auxanometers)

In November and December plots irrigated with two lines of tape per plot (W1) extended only slightly more rapidly than those irrigated less often with only one line of tape (W2). In January PER was similar in both treatments and in February W2 plants grew more rapidly than W1 plants (Fig. 1).

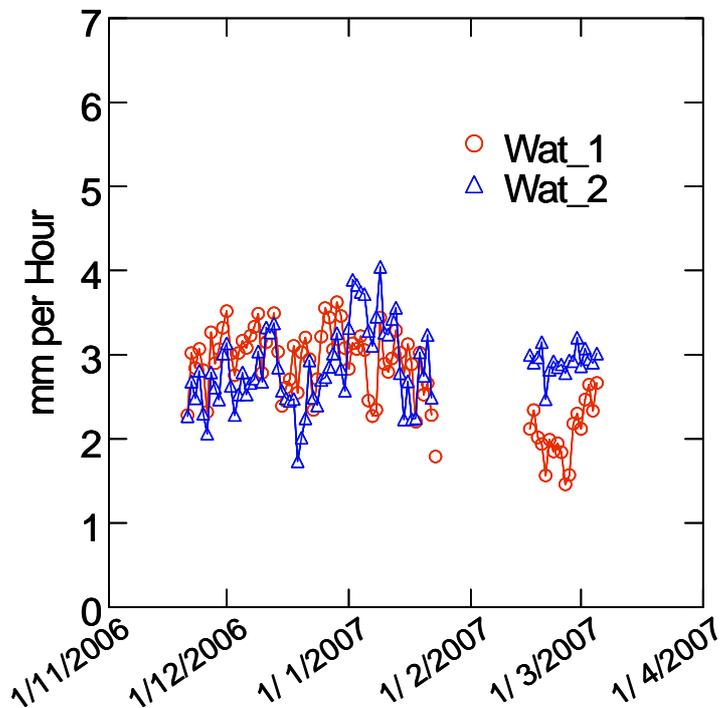


Figure 1 Daily mean plant extension rate (PER) in plots 14 (W1) and 15 (W2) both KQ97-4606.

Lodging ratings

Prior to mid June 07 the trial was not badly lodged apart from four plots, two each of KQ97-2782 and MQ77-340 (both with known lodging tendencies) and three of W1 and one of W2 thus lodging was only partly attributable to differences between clones and irrigation treatment. Plot positioning seemed to be more important possibly because of the prevailing winds from the south east (Fig 2). The lodging rating varied over three dates (March to June) but not consistently reflecting the difficulty in assessing lodging objectively and how little lodging changed over this period (Fig 2).

Despite the variation in lodging score the mean score over three dates was significantly different for the clones with the two lodging types rating higher than the others (Table 1, Fig. 3). The effects of irrigation and the irrigation x clone interaction were not significant.

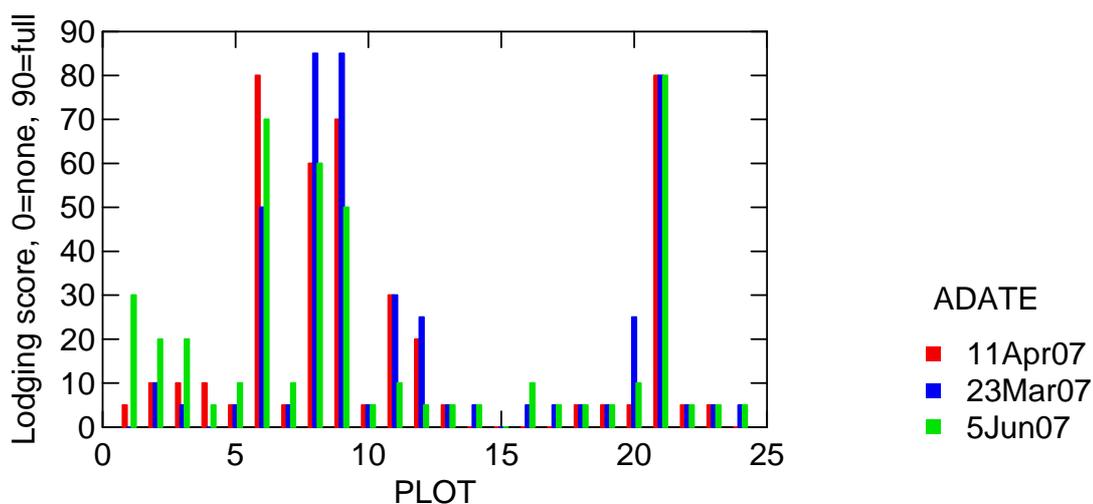


Figure 2. Lodging rating on three occasions for each of the 24 field plots

Table 1. Analysis of Variance for mean lodging score for each plot

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Irrigation	1028.912	1	1028.912	2.656	0.125
Clone	4910.629	3	1636.876	4.226	0.025
Rep	2327.083	2	1163.542	3.004	0.082
Irrig x clone	1881.803	3	627.268	1.619	0.230
Error	5422.917	14	387.351		

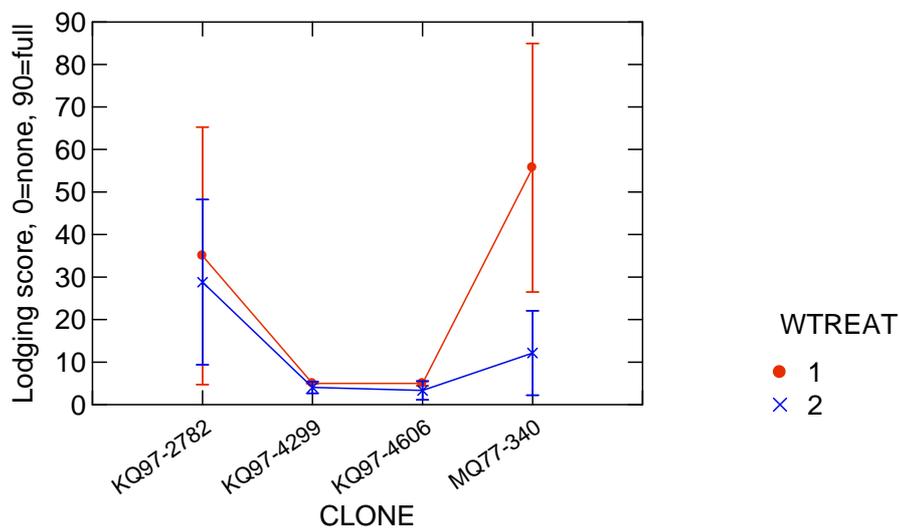


Figure 3. Mean lodging rating over three occasions for four clones

Stalk height

Stalk height was measured in rep 3 only on 16 February and in all plots on 23 March and was considerably greater in the W1 than the W2 treatment in February which was surprising given the similarity between irrigation treatments in the PER data. Irrigation treatment differences were small in March.

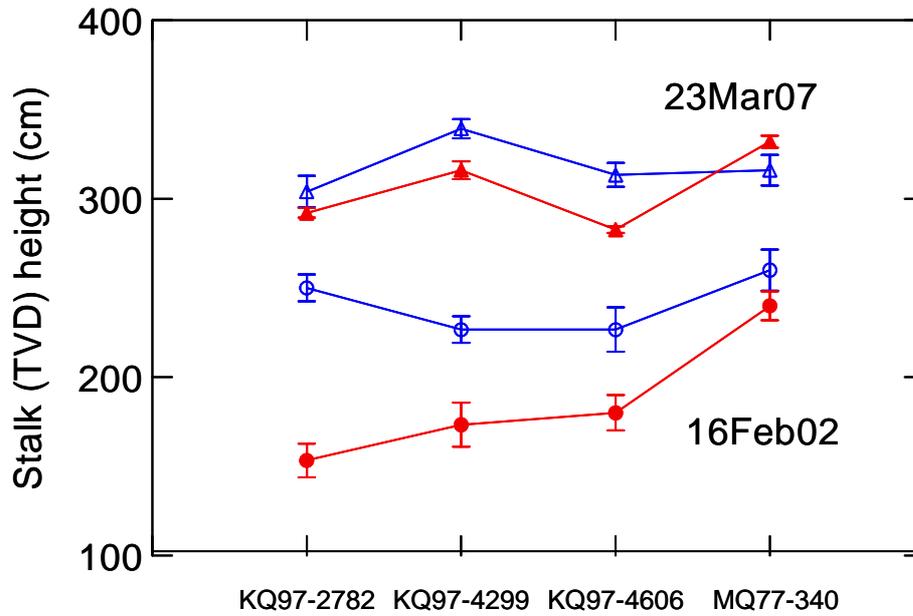


Figure 3. Stalk height measured on two occasions in W1 (○) and W2 (●) treatments and in four clones.

Conclusion

At the end of the wet season differences in stalk height and lodging between irrigation treatments were insufficient to test the hypothesis that erect crops are able to catch up with lodged crops after the wet season. Lodging differences between clones were significant but the clones did not interact with the irrigation treatment as was hoped. It was decided to harvest the trial as early as possible in June in order to allow sufficient time in the ratoon crop to establish height and lodging differences before the next wet season.

Appendix 9

Choice of clones for the lodging experiment

For the lodging trial at Kalama (to be planted on 10/7/06) , we considered clones of the K97 family since these have a wide range of lodging tendencies as well as a wide range of sucrose contents as required for the selection of clones for the TPF sucrose accumulation work. In the tables below, clones in warm colours were considered for erect types and those in cool colours for lodging types.

K97-4606 and KQ97-4299 are the final selections for erect types and KQ-2782 and KQ97-4462 for the lodging types. The final selection was based on consistency in lodging rating, reasonable stalk height and node numbers. It also turns out the one clone of each pair has high sucrose and the other has low sucrose. K97-4606 (erect) and KQ97-4462(lodged) have high sucrose and KQ97-4299(erect) and KQ-2782(lodged) have low sucrose.

Table 1 Lodging ratings on two dates and in two methods on the second date. Ratings were done by GIB on all plots on both dates. On the second date only the sampled plots (one rep) were rated by MFS (ID correct). ID of all plots rated by GIB on 22/3/06 (ID cannot be guaranteed because of difficulty in locating plot boundaries).

Clone	Lodge 15/2/06	Clone	Lodge 22/3/06 (1)	Clone	Lodge 22/3/06 (2)
KQ99-1318	0	KQ99-1318	5	KQ01-3020	1
KQ99-1387	1.5	Q183	10	KQ01-3051	1
KQ97-4299	2	KQ01-3264	10	KQ97-4606	1
KQ01-3060	2	KQ01-3051	10	KQ99-1292	1
KQ97-6765	2	KQ97-4299	25	KQ97-2835	2
KQ01-3105	2.5	KQ01-3020	30	KQ97-4299	2
KQ01-3020	2.5	KQ01-3105	35	KQ97-6765	2
KQ99-1362	3.5	KQ99-1355	35	KQ99-1387	2
KQ99-1292	3.5	KQ97-4606	40	KQ01-3060	3
KQ97-4606	3.5	KQ97-2599	40	KQ01-3177	3
KQ99-1446	3.5	KQ99-1387	40	KQ97-2599	3
KQ99-1501	3.5	KQ99-1292	45	KQ97-6677	3
KQ01-3264	3.5	KQ01-3060	45	KQ99-1355	3
KQ01-3051	3.5	KQ97-2772	55	Q127	3
KQ97-5080	3.5	KQ99-1446	55	KQ99-1446	4
Q183	5	KQ97-6765	55	KQ99-1501	4
TELLUS	5	TELLUS	75	TELLUS	4
KQ01-3115	5	Q117	75	KQ97-5080	5
KQ01-3225	5	Q180	75	Q117	6
KQ98-673	5	KQ97-5080	75	Q180	7
KQ97-2599	5	KQ01-3225	80	KQ01-3225	8
KQ99-1355	5	KQ97-6677	80	KQ97-2772	8
Q180	5	Q138	85	KQ99-1317	8
KQ97-2835	5	KQ01-3115	85	KQ01-3105	9
KQ01-3177	5	KQ01-3177	85	KQ01-3115	9
KQ97-2772	6	KQ99-1362	90	KQ97-2782	9
KQ99-1317	6	KQ99-1317	90	KQ97-4462	9
KQ97-6677	6	KQ97-2782	90	KQ98-673	9
Q117	7.5	KQ99-1501	90	KQ99-1362	9
MQ77-340	7.5	KQ98-673	90	MQ77-340	9
Q138	10	MQ77-340	90	Q138	9
KQ97-2782	10	Q127	90	KQ01-3264	.
Q127	10	KQ97-2835	90	KQ99-1318	.
KQ97-4462	10	KQ97-4462	90		

Table 2. Stalk height, population and nodes stalk on 22/3/06 ranked from high to low

Clone	Hght (cm) 22/3/06	Clone	Stalks/130 cm 22/3/06	Clone	Nodes/ stalk 22/3/06
Q138	342	Q138	30	KQ97-4462	23.2
TELLUS	316	KQ97-2599	25	KQ97-4606	21.8
MQ77-340	315	KQ97-6765	23	KQ97-5080	21.2
KQ01-3115	315	KQ01-3225	21	TELLUS	19.6
KQ97-2835	313	KQ97-4299	21	KQ97-6677	19.6
KQ99-1362	311	Q117	20	KQ99-1355	19.4
KQ99-1355	306	KQ97-4462	20	KQ99-1317	19.4
Q180	300	KQ01-3060	20	KQ97-4299	19
KQ97-4462	297	KQ97-5080	19	KQ97-2835	19
KQ01-3177	291	KQ01-3115	18	KQ97-2782	19
KQ98-673	290	KQ01-3264	18	Q180	18.6
KQ97-5080	290	KQ99-1387	18	MQ77-340	18.6
KQ97-4606	283	KQ99-1355	18	KQ01-3177	18.4
KQ97-2782	281	KQ99-1446	18	Q117	18.2
KQ97-4299	280	KQ01-3051	18	KQ97-2599	18.2
Q127	279	KQ97-2835	18	KQ99-1446	18
KQ01-3060	279	KQ01-3177	17	KQ98-673	17.8
Q117	277	KQ99-1501	16	KQ99-1362	17.6
KQ97-6677	275	TELLUS	16	Q138	16.8
KQ99-1317	271	KQ01-3105	15	KQ99-1318	16.6
KQ97-2599	262	KQ97-6677	15	KQ99-1501	16.6
KQ99-1446	261	Q127	15	KQ97-6765	16.2
KQ01-3105	252	Q180	15	KQ01-3115	16.2
KQ97-6765	247	MQ77-340	15	KQ99-1292	15.8
KQ99-1501	244	KQ97-2782	15	KQ01-3060	15.8
KQ01-3225	240	KQ99-1317	14	KQ99-1387	15.4
KQ01-3020	230	KQ99-1362	14	KQ97-2772	15.4
KQ99-1318	226	KQ97-2772	14	Q127	15.2
KQ97-2772	225	KQ97-4606	13	KQ01-3020	14.8
KQ99-1387	218	KQ98-673	13	KQ01-3105	14.2
KQ01-3051	207	KQ01-3020	12	KQ01-3051	14.2
KQ99-1292	187	KQ99-1318	12	KQ01-3264	13
KQ01-3264	169	KQ99-1292	11	KQ01-3225	12.6
				KQ97-4462	23.2

Table 3 Sucrose % of basal 75 cm stalk on 22/3/06 and of whole stalks on 15/6/06

Clone	Sucrose % FW basal 75 cm (22/3/06)	Sucrose % FW (15/6/06)
KQ97-5080	2.73	14.5
KQ97-6677	2.54	9.6
KQ01-3177	2.47	15.0
KQ97-4462	2.45	10.2
KQ97-4606	2.32	12.1
KQ99-1317	2.24	10.0
Q117	2.11	13.0
Q138	2.05	
TELLUS	2.03	
KQ01-3060	2.01	
KQ98-673	2.00	
Q127	1.97	
KQ01-3105	1.96	
KQ99-1501	1.95	
Q180	1.84	
KQ99-1362	1.71	
KQ01-3225	1.71	
KQ01-3115	1.70	
KQ97-4299	1.63	
KQ99-1355	1.62	
KQ01-3264	1.52	
KQ01-3051	1.51	
MQ77-340	1.48	
KQ99-1292	1.39	
KQ99-1318	1.36	
KQ99-1446	1.35	6.4
KQ01-3020	1.26	9.6
KQ97-2835	1.20	9.4
KQ99-1387	1.17	
KQ97-2599	0.97	6.8
KQ97-2782	0.97	
KQ97-2772	0.64	
KQ97-6765	0.43	7.5

Appendix 10

Report on Clone x Lodging with irrigation experiment, ratoon crop (07/08) CSR Kalamia Estate

This experiment was designed to assess whether irrigation management favours certain clones in selection trials more than others. It is possible that clones prone to lodging will be selected preferentially when irrigation is used sparingly because lodging is delayed compared to a fully irrigated and more commercial situation. The experiment is also designed to assess the capability for erect but retarded crops to catch up with more advanced but lodged crops after the wet season. Clones that are more prone to lodging should benefit more from the delayed growth pattern.

Treatments

Cultivars

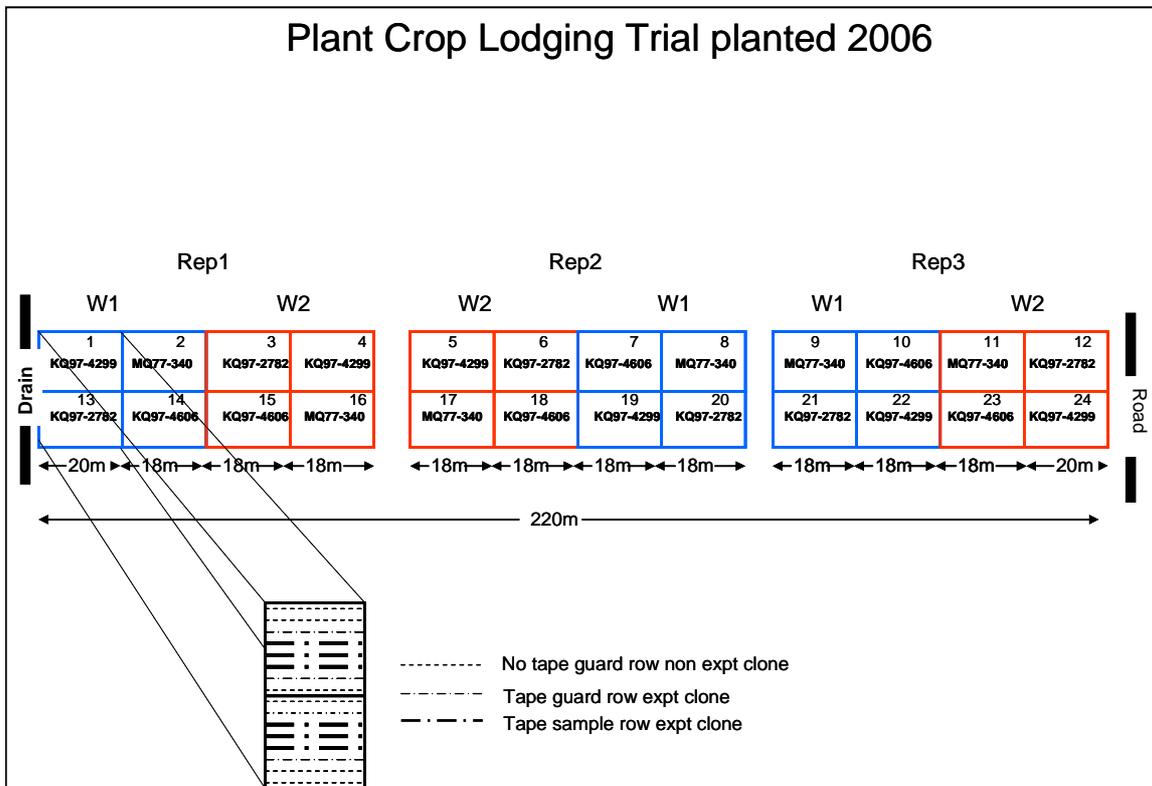
- a. High lodging X 2
- b. Low lodging X 2

Irrigation regimes:

- a. Frequent irrigation to encourage stalk elongation and lodging (W1, wet)
- b. In frequent irrigation to delay stalk elongation and lodging (W2, dry)

Design

The design was a split plot with three replications; whole plots for irrigation treatment and sub-plot for clones.



Choice of clones

Choice of clones for the experiment was based on lodging ratings reported in milestone 4 and on the quality of seed available in the propagation plots. KQ97-4299 and KQ97-4606 ranked

third as the most erect clones in at least one of the lodging assessments of the propagation plots and MQ77-340 ranked fourth and KQ97-2782 second as the most lodged of the 34 clones and cultivars.

The plant crop was harvested on 30 June 2007 and normal estate fertiliser was applied soon after.

Irrigation

The whole site was flood irrigated on 15 July, 2, 27 September and 15 October. From mid-October to mid-April, wet and dry treatments were irrigated independently using WaterSense which calculates a number of attributes of the soil water-plant-atmosphere continuum including soil water deficit, demand for water by the atmosphere and supply of water by the roots. The wet treatment was irrigated when the soil water deficit was about 60 mm as determined in WaterSense and the dry treatment was irrigated when the soil dried out such that only 50-75% of the daily water requirement could be supplied by the roots. Irrigation was applied to each treatment independently through a trickle system fitted with flow meters. Two lines of emitter tape delivered 24 mm per irrigation to wet plots and one line of tape delivered half that amount to dry plots. No runoff was observed over the 8 hours required to deliver these amounts. The trickle tape was removed after mid-April and all plots were furrow irrigated when the soil water deficit reached 60 mm as for the wet (unlimited irrigation) treatment.

The wet treatment received a total of 1152 mm irrigation plus 1285 mm rain and the dry treatment 727 mm irrigation plus the 1285 mm rain (Fig. 1).

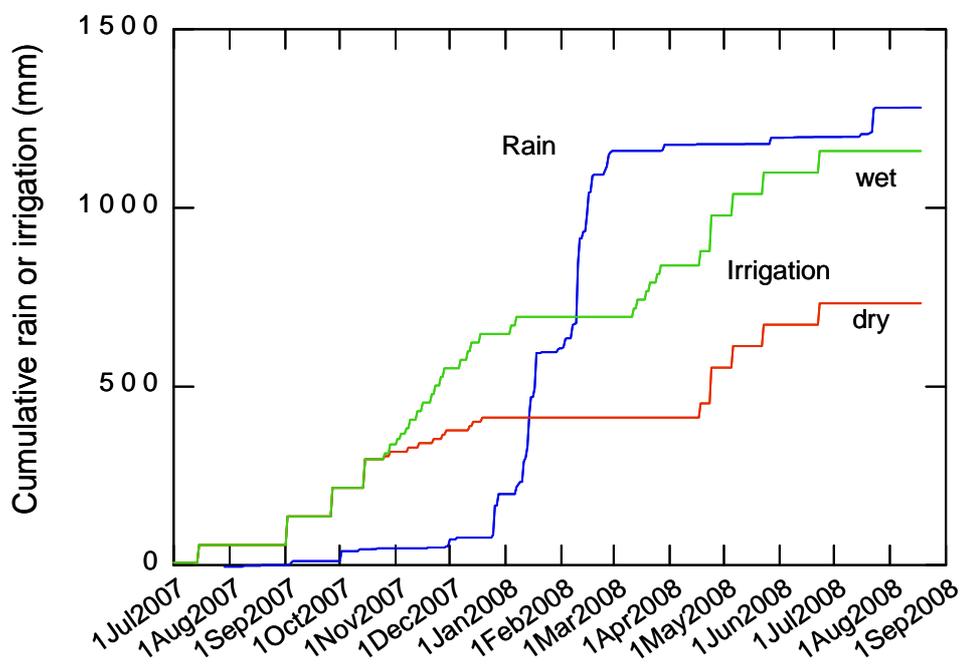


Fig. 1. Cumulative rainfall and net irrigation for the duration of the experiment

Measurements

Plots were rated regularly for stalk height, lodging angle, canopy development and flowering. Lodging was judged by the angle from the vertical of stalks at the base. This was often variable within a plot so stalks were grouped mentally into categories which were rated separately and a weighted average obtained.

Sample harvesting was conducted on two occasions, once after the end of the irrigation treatment period (1-3 April 2008) and from 18 to 20 August 2008, shortly before machine harvesting. Ten adjacent stalks were cut at the base and bundled before removing all plant material from 16.2 m² of net plot area. All material was weighed and the 10 stalk sample was then partitioned into leaf blades and sheaths and stalks while counting green leaves and internodes. Dry matter content was determined for each part and sucrose content for the stalks, following the method described by Muchow *et al.* (1993).

Results

Ratings

Lodging after heavy rains in January was more severe in the wet than the dry treatment particularly for the two clones MQ77-340 and KQ97-2782 which are prone to lodging (Fig. 2). Stalks were about 50 cm shorter in the dry than the wet treatment at this time and this no doubt helped the crop in the dry treatment to withstand January storms by remaining considerably more erect than was the case in the wet treatment (Fig. 2). In wet plots, stalks of MQ77-340 were estimated to be about 50 cm taller than those of KQ97-4606 and this difference could partly explain the different propensity to lodge in this clone. However KQ97-2782 was also about 50% lodged in April and May but did not have particularly long stalks. Stalks of the dry treatment were of similar height by mid March apart for one clone (KQ97-4606). Thus the return to a normal unlimited irrigation schedule after the wet season appeared to be working as intended to help the delayed but mostly erect crop to 'catch up' to the well watered crop (Fig. 2).

Canopy development during November and December was reduced substantially in MQ77-340 by reduced irrigation but the effect on canopy development for the other clones was not great (Fig. 2).

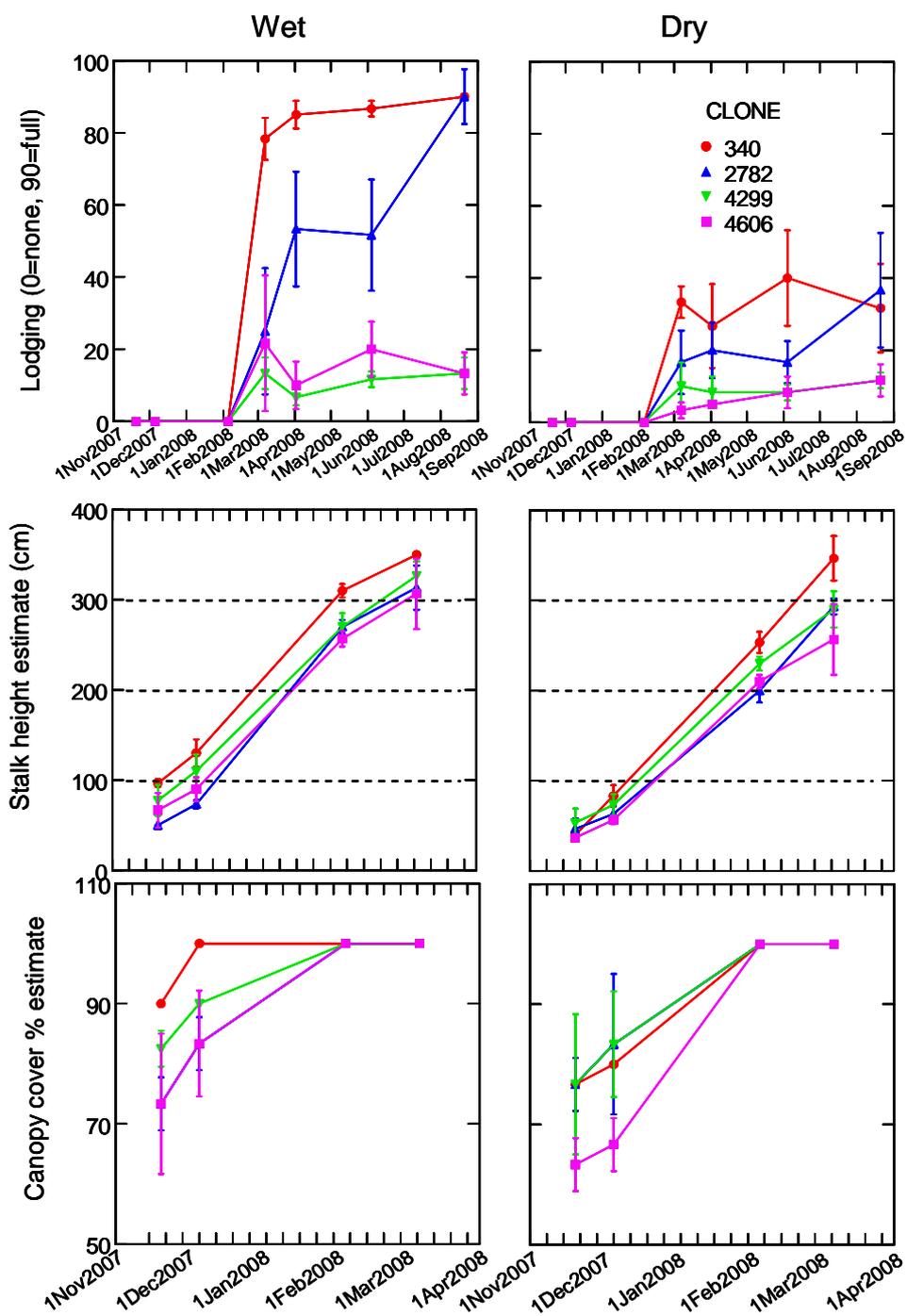


Fig. 2 Ratings of lodging angle (from vertical), stalk height and canopy cover during the period of most rapid height growth for four clones and two irrigation treatments.

Plant sampling and ratings in April and August (Fig 3)

Flowering was fairly severe in MQ77-340 and KQ97-2782 when ratings were conducted in August and was not affected by irrigation (Fig 3). Specific leaf area (SLA) was high in all clones at the time of the first sampling and decreased considerably for MQ77-340 during the April to August period when this clone lodged completely in both irrigation treatments. Irrigation to a 60 mm soil water deficit while not excessive exacerbated lodging in MQ77-340 and KQ97-2782 but not at all in KQ97-4299 and KQ97-4606. The interaction was highly significant ($P < 0.001$) thus achieving the aims of this experiment. Earlier experiments were not so successful. The number of green leaves per stalk reflects both the degree of water stress and lodging. It is interesting that green leaves per stalk were reduced significantly by irrigation in April and were increased significantly in August, the two mechanisms working in opposition.

Stalk population was considerably higher in KQ97-4606 than the other clones and was similar for both sampling dates except for KQ97-2782 which suffered significant stalk loss over the April to August period. Nevertheless stalk populations were reasonably high for Australian germplasm and >12 stalk/m² for KQ97-4606 was exceptional particularly for a row spacing of 1.8 m and a first ratoon crop.

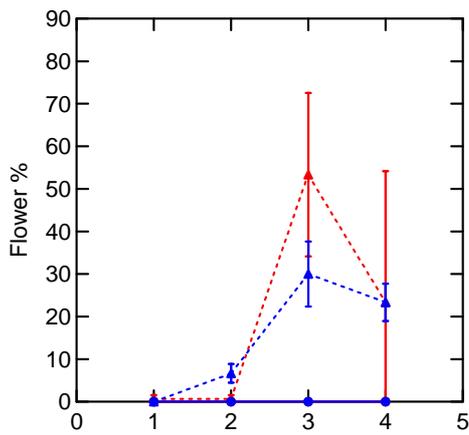
The fraction of biomass in millable stalk increased substantially over the April to August period and was increased significantly by increased application of irrigation. The clones also differed significantly in this regard with about 90 % of biomass in stalks of KQ97-4299 and MQ77-340 in August after liberal irrigation amounts.

Stalk dry matter content increased substantially from April to August reaching levels as high as 35% in KQ97-4299 which is very high for whole stalks of sugarcane. Clone differences were significant on both sampling dates. Stalk dry matter content was higher in the wet than the dry irrigation regime in April possibly because of retarded stalk growth and limited repartitioning to sucrose which is often found with moderate water stress. The lack of such repartitioning (ripening) was probably due to the frequent but limited (12 mm) irrigations in the 'dry' treatment

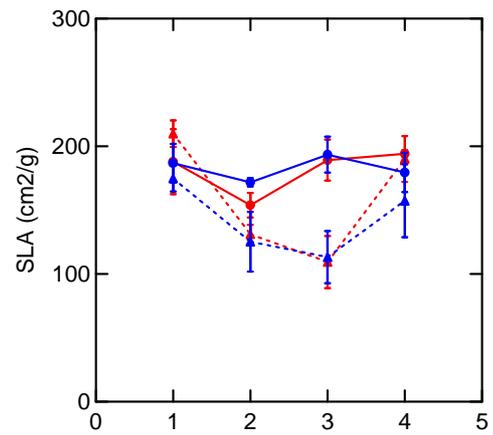
Total biomass, stalk dry and fresh mass (cane yield) were all reduced significantly in April by restricted irrigation during October to March. However the apparent differences in these yield attributes were not significant for either irrigation or clones in August. Between April and August total biomass increased 27% in the wet regime and 39% in the dry regime and stalk dry mass increased 50 and 62% in wet and dry regimes respectively. Thus biomass accumulation in the dry treatment was 44 % greater than for the crop receiving unlimited irrigation throughout its development.

Clone differed significantly in regard to sucrose content on both fresh and dry matter bases, in April and in August. Sucrose content was reduced in the limited irrigation treatment in April but not in August.

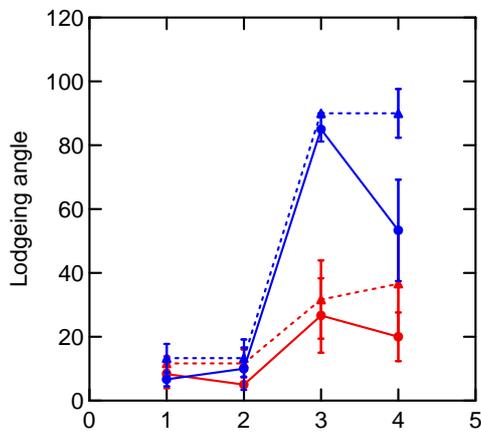
Sucrose yield was highest in KQ97-4299 and lowest in KQ97-2782 in April and was reduced in the limited irrigation treatment in April but neither clone or irrigation treatment effects were significant in August.



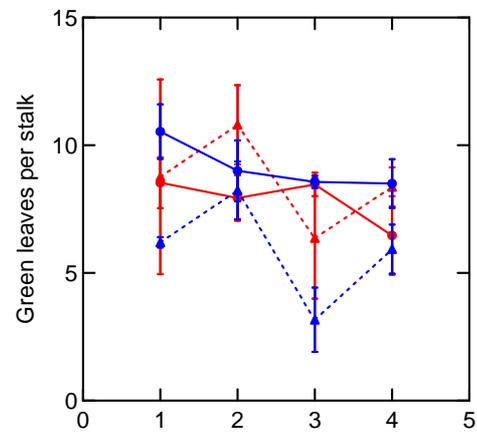
P=0.005 for Clones (20Aug)



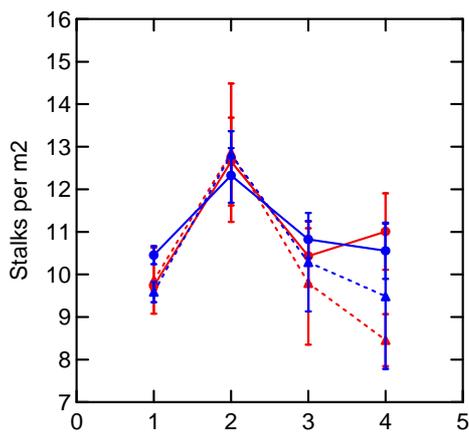
P<0.001 for Clones (19Aug)



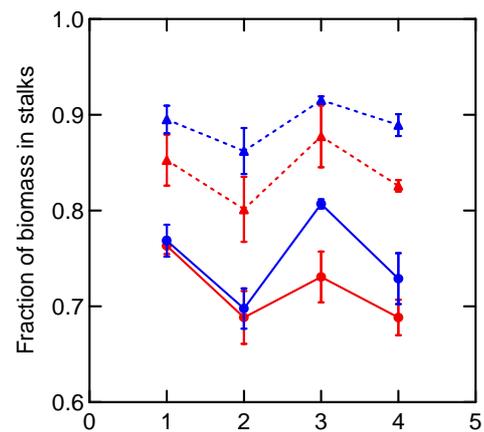
P<0.001 for Clones, Irrigation and interaction on both dates



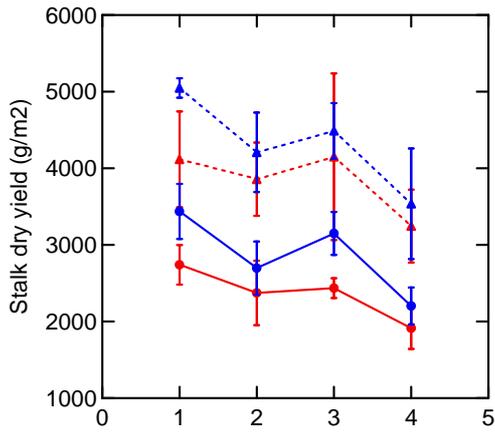
P<0.05 for Irrigation on both dates and for Clones on 19Aug.



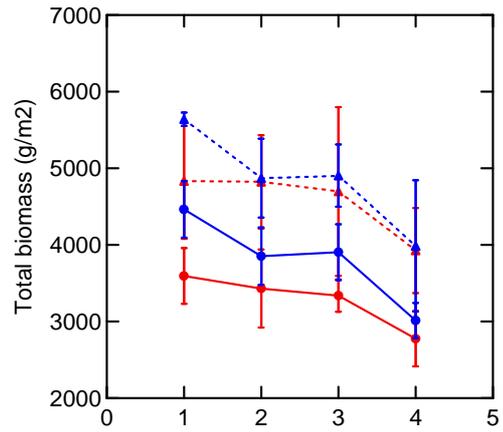
P<0.005 for Clones on both dates



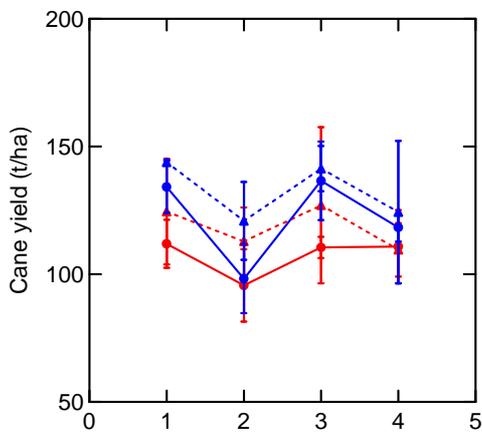
P<0.01 for Clones and Irrigation on both dates



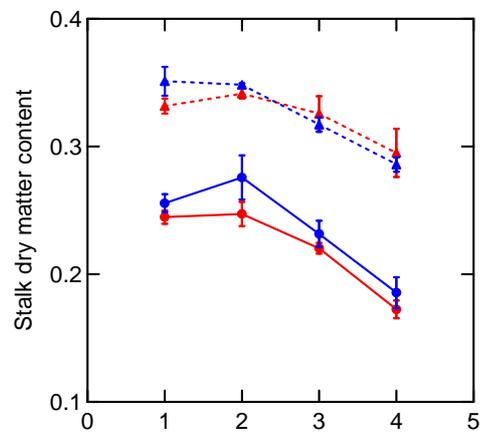
P<0.01 for Clones and Irrigation on 1Apr.



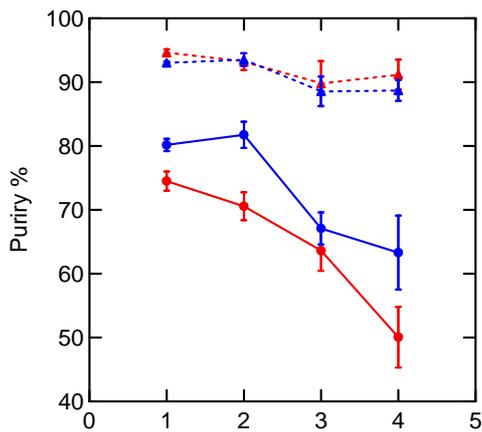
P<0.02 for Clones and Irrigation on 1Apr.



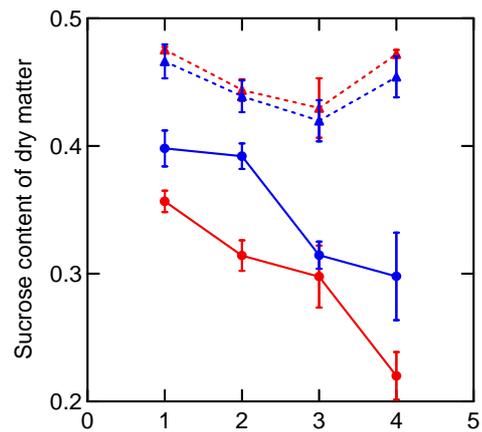
P<0.05 for Clones and Irrigation on 1Apr



P<0.001 for Clones on both dates P=0.005 for Irrigation on 1Apr.



P<0.001 for Clones and Irrigation on 1Apr and P=0.017 for Clones on 19Aug.



P<0.001 for Clones and Irrigation on 1Apr and P=0.003 for Clones on 19Aug.

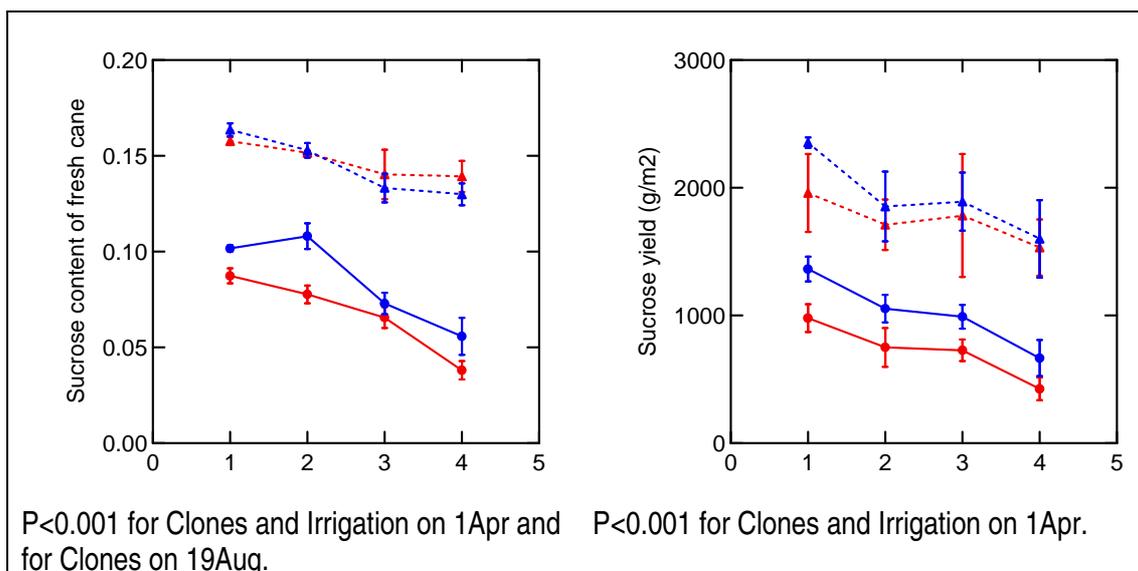


Fig. 3. Yield components of four clones determined in April (solid lines) after differential application of irrigation to the wet (blue) and dry (red) treatments, and in August (dotted lines) after all plots received the same liberal irrigation from 1 April onwards,

Discussion

The experiment was highly successful in that lodging was ‘controlled’ through selection of clones for lodging proneness from a segregating population and through careful control of irrigation. These treatments resulted in the desired combination of fully lodged prone cultivars, erect prone cultivates and erect non-prone cultivars at the end of the wet season. We could then observe growth in many yield components from April to August while all plots were irrigated equally. Cane yield increased about 10% over this period regardless of clone or irrigation treatment. Dry matter content of stalks increased markedly over this period resulting in large increases in total and stalk dry biomass. However the increase was not linked to degree of lodging whether this was assessed at the beginning or end of the April to August growth measurement period. Contrary to expectations lodging due to either genotype or irrigation treatment had no measurable effect on any yield component in this experiment. This is surprising particularly for MQ77-340 which was fully lodged by 1 April and yet continued to accumulate biomass and sucrose at about the same rate as the other clones.

The effect of irrigation treatment on yield components (total biomass, cane yield and sucrose yield) was significant when sampling was done at the end of the treatment period in April but no differences were significant in August. The loss in sucrose yield due to withholding irrigation was 29% in April and only 9% in August if we accept that this is loss is real but not measurable. This slight (if any) yield advantage for the liberal irrigation regime came at the cost of an additional 558 mm (5.6 ML/ha) irrigation. This experiment proved yet again that at least in some soils Burdekin growers could reduced irrigation amounts considerably without significant yield loss and it also proved that lodging can be delayed by withholding irrigation to some extent before the wet season. However the benefits in delaying lodging were not as clear as was expected from controlled experiments by Singh et al (2002). Berding and Hurney (2005) described lodging as a chaotic phenomenon so we could expect the results of controlled lodging experiments to be highly variable depending on the way in which the crop succumbs to lodging. We could argue from these results that it would be best to delay lodging by controlled limited irrigation to limit the risk of large yield losses associated with lodging as determined by Sing et al (2002) and as supported by our earlier results (Inman-Bamber et al, 2004).

The irrigation treatments applied in this experiment were based on the WaterSense model and are therefore repeatable. However a trickle system would be required to apply the limited but frequent irrigations as in this experiment. No doubt an effective regime could be worked out for a furrow irrigation system.

Conclusions

This experiment did not support the hypothesis that irrigation management favours certain clones in selection trials more than others. It did not indicate that clones prone to lodging will be selected preferentially when irrigation is used sparingly because lodging is delayed compared to a fully irrigated and more commercial situation. However the experiment did indicate that erect but retarded crops can 'catch up' with more advanced but lodged crops after the wet season but this did not favour clones that were more prone to lodging. The capacity for sugarcane to recover from mild water stress during the rapid period of growth adds more evidence for the capacity for water savings in the Burdekin should this become necessary.

Appendix 12

DIRECTIONS FOR R&D AND CANE GROWING FROM AN INTERNATIONAL REVIEW ON SUGARCANE PHYSIOLOGY

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Abstract

Sugarcane physiology has lagged well behind that of other crops for nearly 40 years. In recognition of this, the Sugar Research and Development Corporation of Australia provided funds for an international workshop on sugarcane physiology in September 2003. The goal of the workshop was to identify the critical gaps in our understanding of sugarcane physiology that, if addressed by research, could lead to advances in the economic and environmental sustainability of sugarcane production. This paper presents some of these gaps and identifies opportunities for increased production and resource use efficiency.

It is likely that current sucrose yield potential will not be substantially increased through current conventional breeding and agronomic approaches, as much as through targeted genetic improvements in areas such as photosynthetic efficiency and partitioning of photosynthates among metabolic pools.

In many cases we are not achieving currently attainable yields partly because of lodging, flowering, suckering, reduced soil health, tiller senescence and stalk death. Selection against some of these traits and mitigating management practices will help to reduce the gap between attainable and actual yields. Productivity can also be increased by better matching cultivars to the environment and by managing them to fully exploit their favourable traits. Models capable of simulating cultivar differences are essential for such advances.

The workshop participants recognised that closer collaboration between molecular biologists, breeders, agronomists and modellers to explore and exploit the interaction of genotype and environment (including management) at crop, plant and molecular levels was more likely to lead to improved industry outcomes than working in isolation.

Keywords: sugarcane physiology, GXE interaction, R&D priorities, collaboration

Introduction

Sugarcane received attention for a short while in breakthroughs on photorespiration and the C₄ photosynthetic cycle (Kortschack *et al*, 1965; Hatch and Slack, 1966). Since then progress in sugarcane physiology at all levels (molecular to crop) has fallen behind that of other major crops. The proliferation of crop simulation models in the 1980s and 1990s led to some basic investigations in order to provide rate coefficients for sugarcane growth models. At about the same time sugar industries in several countries started investing in biotechnology for sugarcane and the investment and intensity of this research has increased remarkably with a consequent reduction in emphasis on ecophysiology. Modelling and molecular technologies took sugarcane physiologists in these two different directions and little research was done outside these two fields. While crop simulation technology was able to help solve some sugarcane production problems, the solution to other issues was frustrated by the lack of basic physiological information. In Australia low yields and low sucrose content in extreme climates (high temperature or high rainfall) could not be explained by models or current knowledge of plant or crop physiology (Wilson and Leslie, 1997; Leslie and Byth, 2000).

The size and complexity of the polyploid genome of sugarcane means that the genetic tools available to sugarcane scientists also lag behind those of model plants and other crops such as *Arabidopsis* and rice. In these plants, the sequence of the whole genome and an increasing number of characterised mutants are available for studying the connection between particular genes, physiology and phenotype. Such tools are not yet available for sugarcane.

In recognition of the comparatively poor state of physiological knowledge in sugarcane and the divergence in the research areas at the molecular to whole crop scales, the Sugar Research and Development Corporation (SRDC) of Australia provided funds for an international workshop on sugarcane physiology, with the theme of integrating knowledge from cell to crop to advance sugarcane production. The goal of the workshop, which was attended by about 40 delegates, was to identify the critical gaps in our understanding of plant and crop physiology for sugarcane that, if addressed by research, could lead to advances in the economic and environmental sustainability of sugarcane production.

This paper presents the highlights of several papers (not all) and major challenges for R&D emerging from the workshop. A case is made for collaborative research across levels of organisation (molecule to mill).

Yield potential and complexity

The paradigm introduced by authors such as Rabbinge (1993) have been challenged. In the past 10 years or more we have thought of production in terms of actual, attainable and potential yields. This paradigm is really an extension of Justus von Liebig's (1803-1873) 'Law of the Minimum', which was about plant nutrition originally, but has been extended to include pests, water, radiation and a host of other factors. Attainable yields were generally thought to be limited by water or nutrition and potential yields by radiation and temperature. The key-note paper by Dr Paul Moore (2005) introduces us to the 'age of complexity'. The great volume of information generated from sequencing the expressed sugarcane genome has not

produced an understanding of what it all means about plant growth and development. “Collecting and analysing the information from even a single high-throughput experiment quickly reveals the complexity and magnitude of effort required to build a sufficiently comprehensive dataset to predict how the plant, or tissue, or cell, or biochemical pathway will perform under every different set of conditions. One can scarcely envisage the magnitude of systematically establishing perhaps 500 different environmental or developmental stages over which to compare the mRNA expression of a genome of 25 000 genes,” Moore (2005). Dr Moore likens our current approach to the study of biological systems to an attempt to build a Boeing 747 by having blueprint specifications only for each of the components. “What is needed in place of a traditional bottom-up approach, i.e. analysing the details of small sub-systems, is a top-down approach of modelling the entire system from the behaviour of its many sub-systems.” “The ultimate goal is to understand the biological system in sufficient detail to enable accurate, quantitative predictions about its behaviour, when we somehow manage to introduce or block the expression of a suite of genes. This will give us the ability to engineer the design of a crop plant predictably. The challenge is to improve our understanding of how plants function at all scales of complexity to such an extent that we can produce models that will predict how a crop will respond to any given genetic manipulation or environmental perturbation,” Moore (2005).

It will be clear from highlights of other papers that we have a long way to go in unravelling the complexities of the ‘molecule to mill production line’. If we bear in mind that the use of knowledge of each component is limited by our understanding of the interaction with other components and levels of organisation, we have a better chance of improving on a centuries-old law (von Liebig’s) that may now be the wrong paradigm for increasing yields and resource use efficiencies.

Gene expression correlated with sucrose accumulation (functional genomics)

An increasing number of studies are using the information in collections of expressed sequence tags (ESTs), partial sequences of genes expressed in the tissue analysed, to study gene expression of many genes simultaneously. Watt *et al.* (2005) concentrated on analysing the expression of genes known to be involved in sucrose metabolism directly and indirectly in studies of the progenitor species of modern sugarcane cultivars. The progenitors did show some reduced expression of some key enzymes. Using a larger collection of sequences, Casu *et al.* (2005) identified 62 genes that displayed differential regulation between low CCS and high CCS progeny (CCS or commercial cane sugar, is a measure of sucrose content in cane). These were separated into various functional classes (see Casu *et al.*, 2001, 2004, 2005) and are presented in Figure 1. Interestingly, only two of the candidate genes (one up-regulated, the other down-regulated) appear to be genes with known functions in carbohydrate metabolism. The others, some of which have no known function, would be missed in a more targeted approach. These experiments illustrate an approach to the identification of genes with expression patterns that correlate with particular traits, in these cases sucrose accumulation. The targeted versus more global approach is driven at least in part by cost and ease of analysis and interpretation of the results. As these techniques become more widely available and the complement of genes available to test becomes more comprehensive and analysis systems develop further, both target and global hypotheses can be tested in the same experiment. The recent announcement from Affymetrix that a sugarcane oligonucleotide chip based on all of

the publicly available sugarcane gene sequences will be available in early 2005, goes part of the way towards this capacity. Alternative techniques for quantifying the relative expression of particular transcripts can also be used for targeted studies. For example, quantitative reverse transcription-polymerase chain reaction (RT-PCR), a way of measuring the amount of the message for gene(s) of interest against an internal standard, has been applied to sugarcane (Iskander *et al.*, 2004).

Watt *et al.* (2005) suggested that identification of transcriptional events regulating storage of sucrose would be better served if expression profiling were conducted in a system that permits experimental manipulation of sucrose levels in a single genotype and examination of potential feedback between source and sink. Studies in manipulated systems and different genotypes are continuing and analyses using some of the systems biology approaches described by Moore (2005) may help elucidate the regulatory processes and networks of genes controlling sucrose accumulation.

The recent work by Casu *et al.* (2005) and Watt *et al.* (2005) demonstrates the strong influence of developmental physiology and environment on gene expression and the danger of making simple conclusions about the importance of genes involved in sucrose metabolism. This underlines the challenge by Paul Moore to improve our concept of the whole system (Jumbo) while working on basic building blocks and processes (nuts and bolts).

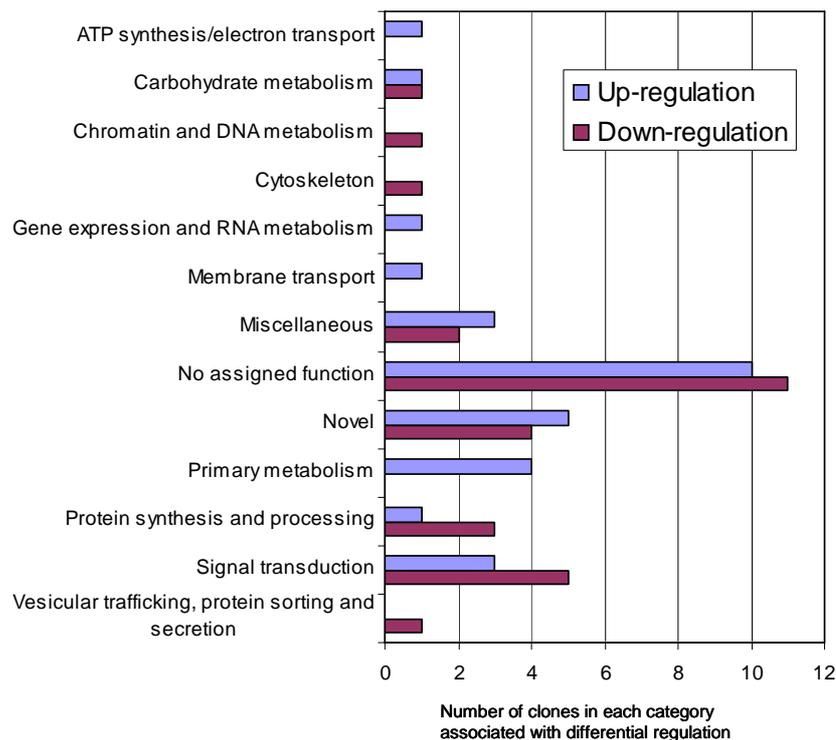


Figure 1. Classification of genes that were up-regulated or down-regulated in high CCS progeny into functional groups. The functional groups are as described in Casu *et al.* (2001, 2003, 2004). Reprinted from *Field Crops Res*, 92, 2-3, Casu *et al.* (2005), with permission from Elsevier

Sucrose storage at the cellular level

“Information on the cellular and sub-cellular location of the proteins that facilitate sugar transport will be immensely valuable in building a complete picture of the pathways and control points,” Rae *et al.* (2005). As the sugarcane stem consists of internodes of different ages and stages of development, it is important to be aware that the metabolic activities are not uniform from one internode to another but will change with development. As the internodes mature, the balance between synthesis, growth and storage of carbohydrates changes. This can be seen in decreased sucrose/hexose ratios and invertase activity and increased expression of sugar transporters in the membranes of older internodes. A comprehensive model of sugar transport and accumulation pathways will need to take these developmental changes into account. Figure 2 shows the possible routes for transport of sucrose into the storage parenchyma. In this model, sucrose is unloaded symplastically from the phloem, potentially moving symplastically throughout the storage tissue by cell-to-cell connections. However, the presence of sucrose in the apoplast together with the need to maintain a gradient for continued unloading suggests that at some point, sucrose exits the symplastic continuum. Sucrose or the products of invertase activity may be taken up by parenchyma cells via membrane transporters and may subsequently cross the tonoplast, the membrane enclosing the vacuole. Enzyme activity may interconvert sucrose and hexoses within each of these compartments. The localisation of the components of this pathway is critical to completion of this model.

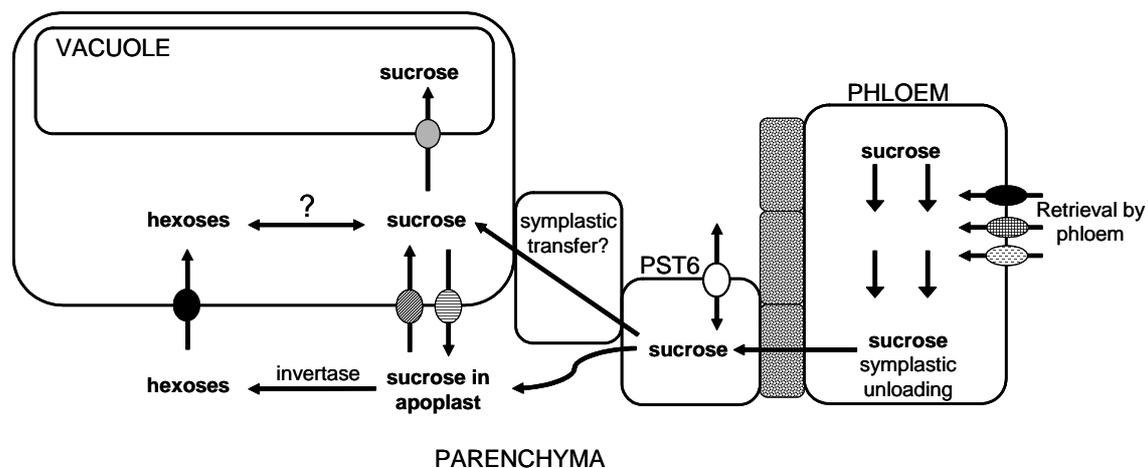


Figure 2. Proposed model of sucrose transport between phloem and parenchyma vacuoles. PST6 = putative sugar transporter no. 6. Reprinted from Field Crops Res, 92, 2-3, Rae *et al.* (2005), with permission from Elsevier.

An understanding of the changes in development would also have utility in understanding ripening and strategies to increase the proportion of mature internodes in harvested stalks. There is clearly synergy to be gained by linking the results of these developmental studies with the more agronomic studies of Singles *et al.* (2005a) described later.

Capture of resources in the soil (sugarcane roots)

The attention received at the molecular and cellular level to study the processes of sucrose accumulation in the stalk has no parallel in other processes or organs. The anatomy of sugarcane root systems was well researched in the 1950s and 1960s (Evans, 1964). Some of the most detailed work was done at the famous underground root laboratory at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, where roots probably still receive more attention than anywhere else in the sugarcane world (van Antwerpen, 1998). From evidence presented in a review of root growth and function (Smith *et al.*, 2005) it is clear that sugarcane can extract water and nutrients from considerable depths and can make use of water upflow from water tables. This knowledge is ignored by and large in management of nutrients and water. Smith *et al.* (2005) urge that research and models acknowledge contributions from deeper in the soil profile which could result in better resource use as well as reduced off-site impacts. There is sufficient evidence to show that roots exert some control on transpiration and assimilation, to focus our attention on the environment in which they live (Davies and Meinzer, 1990). Data presented by Bell and Garside (2005) indicate that improved soil health in the form of structure, chemistry and biology, leads to improved assimilation even when the supply of soil water and nutrients is thought to be adequate. These authors raise the possibility of a poor rooting environment (e.g. compaction, parasitic biota) requiring excessive photoassimilate to support a turnover of root biomass as well as reduced capacity to exploit all of the soil volume.

Better knowledge of root system responses to the soil environment could therefore help to address constraints on productivity and underpin development of farming systems for sugarcane which are more successful at sustaining soil health.

Questions raised about assimilate partitioning between roots and shoots deserve answers. It is possible that high radiation use efficiency, measured in terms of above ground biomass, has come at the expense of roots during breeding and selection over many years. Radiation use may have improved, but inadvertently at the cost of lower capacity for the crop to utilise available water and nutrients for production. Such an effect would result in higher input requirements, particularly for water and mobile nutrients, as well as greater off site impacts (Smith *et al.*, 2005). They therefore suggest that radiation use efficiency and water and nutrient utilisation are compared in old and new cultivars to determine if our selection programmes are leading away from efficient resource use in sugarcane farming systems. In Australia there is a lack of drought resistance in modern cultivars and there is no attempt to select for this trait. Deeper rooted and drought resistant cultivars are required, at least on a limited scale, for irregular droughts which can be very severe (Smith *et al.*, 2005).

Water use and tolerance to drought (water relations)

Although sugarcane is grown mainly in the tropics and to a lesser extent in the sub-tropics, water often limits cane production and reinforces the law of the minimum. The high correlation between transpiration and photosynthesis (Monteith, 1986) also reinforces the dominant role that water plays in production. A review on water relations in sugarcane (Inman-Bamber and Smith, 2005) while not detracting from the dominant role of water, provides some opportunities for increased production and reduced water use and highlights the strong interaction between genotype and response to water deficits. Sugarcane can withstand some degree of water stress

without affecting biomass and sucrose accumulation. Expansive growth (stalk volume, leaf area) is highly sensitive to water stress which is probably unavoidable in many situations, even if irrigation water is unlimited.

Irrigation

The resilience of sugarcane to water stress during early expansive growth was demonstrated by withholding irrigation for a period of almost five months during winter in the Burdekin (Robertson *et al.*, 1999). This treatment reduced leaf area index (LAI) substantially (from 1.8 to 0.9), but cane and sucrose yield at harvest were unaffected. Irrigating when stalk elongation rate falls to 50% of potential is recommended in Australia (Holden, 1998) to ensure that water does not limit biomass or sucrose yield. However, Inman-Bamber (2004) found that biomass was not reduced until stalk elongation rate dropped to less than 30% of the potential rate. The differential response of photosynthesis and expansive growth to water stress provides an opportunity for controlling 'source-sink' gradients that influence translocation of sucrose from leaves to stalks. Two experiments were cited where large increases in sucrose content were achieved during gradual imposition of water stress (Inman-Bamber and Smith, 2005). In one case this led to a 3.6 t/ha increase in sucrose yield (Inman-Bamber, 2004).

Genetics

From the review (Inman-Bamber and Smith, 2005) it was clear that there is considerable variation among sugarcane cultivars in response to water deficits. While osmotic adjustment occurs in sugarcane, the reported adjustments (0.18 to 0.50 MPa) were not as large as those reported for other crops (e.g. rice cultivars, 0.24 to 1.90 MPa, Chandra Babu *et al.*, 2001) and there was little evidence for variation in this trait amongst genotypes. Drought avoidance mechanisms such as leaf rolling, leaf shedding and stomatal closure are prevalent and vary considerably between genotypes. Responses to water stress in root and leaf conductances varied considerably between the few genotypes considered (Saliendra and Meinzer, 1989). As far as is known, none of these traits has been deliberately bred or selected. Information about drought response amongst cultivars is generally gained after they have been released for commercial production. Nevertheless, physiology and 'experience' of drought resistance amongst genotypes have always matched where both are known. This provides confidence that measured traits such as early stomatal closure and leaf shedding confer drought resistance in a manner that is commercially significant to growers.

The limited work on ^{13}C discrimination (Δ) in sugarcane should encourage breeders to investigate this trait for breeding and selection of genotypes that are more water efficient and have less leakage of CO_2 from the bundle sheath. The correlation between Δ and transpiration efficiency (ϵ) found in 30 lines of *Sorghum bicolor* (Henderson *et al.*, 1998) should also serve as encouragement for this approach.

There is sufficient evidence from the review on water relations to look for innovative ways of using water better and for breeding and selecting for higher WUE. Major breakthroughs in WUE in C3 plants (wheat, Condon *et al.*, 2004) are inspiring, but cannot be extrapolated directly to sugarcane for which WUE is already high compared

to wheat. Water savings are possible during both early and late stages of development and possibly during rapid stalk elongation as well. It may be possible to constrain stalk height, reduce lodging and enhance sucrose content by careful control of water relations by irrigation. Genetic variation in Δ , ϵ and rooting depth would also be worth investigating.

Crop development

Leaf appearance

The weight of evidence reviewed by Singels *et al.* (2005a) indicated the interval in thermal time between the appearance of successive leaves (phyllochron) gets progressively longer as the crop develops. This contradicts an earlier hypothesis that there is a distinct change in crop phenology after the 17th leaf appears (Inman-Bamber, 1991). Leaf appearance rate was the same for all shoots regardless of when they emerged.

Leaf size

Leaf size distribution along the stalk varied with cultivar and start date (Singels *et al.*, 2005a). The maximum size was reached at a higher leaf position when crops were planted or ratooned in winter (June) compared to those starting in summer (Figure 3).

Singels *et al.* (2005a) developed the following principles for leaf growth from their review. Each leaf has a predetermined phyllochron in which to complete its expansive growth. This varies predictably with leaf position and cultivar. Final leaf size is determined by photosynthetic activity (source strength) during the phyllochron. Source strength is the product of source size (area of fully expanded green leaves on a given shoot) and source activity determined by environmental factors such as radiation and water status. This explains why leaves become progressively larger until the source size stabilises or until the genetically determined maximum leaf size is reached. It further implies that the size of the first few leaves has a profound effect on the size of all subsequent leaves. This characteristic is cultivar specific.

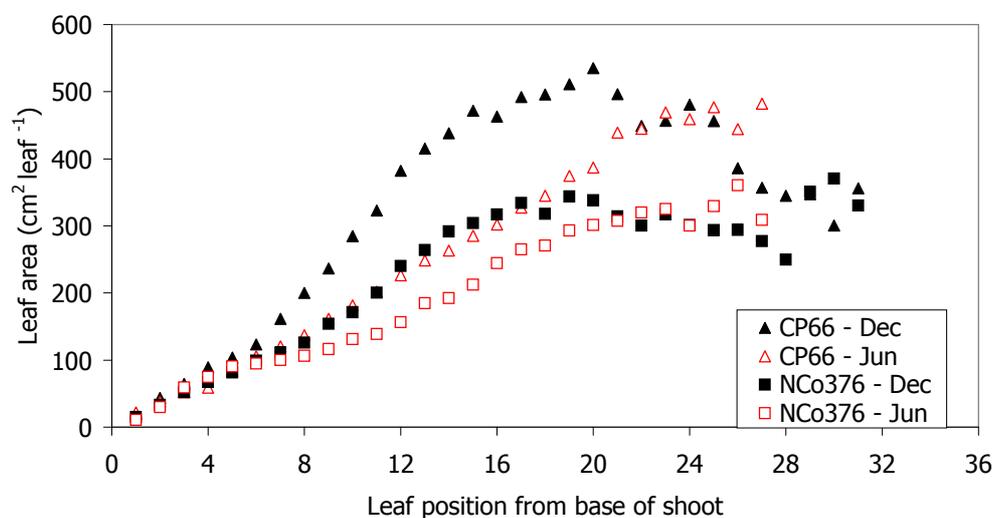


Figure 3. Mean area of successive leaves (bottom to top) on stalks of NCo376 and CP66/1043, ratooned in June or December at Mount Edgecombe. Reprinted from *Field Crops Res*, 92, 2-3, Singels *et al.* (2005a), with permission from Elsevier.

Stalk dynamics

The crop canopy is obviously comprised of the leaves on all of the stalks, however, there has been some confusion about population density as a factor determining yield. The large yield increases obtained in ‘high density planting’ experiments in Australia could not be repeated in similar experiments both in Australia and South Africa (Bell and Garside, 2005). Good eventually came out of the confusion, which led to an understanding of the components that contributed to large yield increases that were claimed. Bell and Garside (2005) reviewed this work, much of it their own. A thinning experiment demonstrated the remarkable ability for sugarcane to compensate for changes in stalk population. Crop biomass was reduced by only 4, 30 and 63% respectively by removing 25, 50 and 75% of the stalks four months prior to harvest. Individual stalk mass often declined when stalk population was increased deliberately through managing row spacing and planting density. The negative correlation between stalk mass and population was however weak under conditions of poor soil health as capacity to fill stalks was limited. Conversely, when soil health was improved through crop rotation or fumigation similar yields were obtained for a range of populations as fewer stalk numbers could be compensated for by larger stalks, unless the crop was severely stressed (Bell and Garside, 2005).

Improving soil health through fumigation or legume break crops increased the primary shoot population by as much as 50%. This resulted in less tillering and a greater contribution of primary stalks to the yield than was the case for unfumigated or continuous sugarcane treatments. As Bell and Garside (2005) showed that there was a greater propensity to lose tillers than primary stalks, they suggested that an avenue to improved crop yields would be to have a higher proportion of primary stalks. Management or breeding strategies that increase the population density of primary stalks and their contribution to yield are likely to increase productivity simply through increasing the average duration for growth for each stalk.

Singels *et al.* (2005a) reported work where viable bud density was varied by changing row spacing and crop class, and was found to be largely responsible for the subsequent rate of shoot appearance and peak shoot population density (Figure 4). Singels *et al.* (2005a) found that tillering began after a lag period of approximately 300°C.d (base 16°C) after emergence of primary shoots. Tillers emerged regularly in relation to thermal-time depending on cultivar (42°C.d per tiller for NCo376). Peak shoot density occurred at a similar thermal-time after crop start (600°C.d) regardless of row spacing, start date or crop class. Thus tiller senescence was not directly linked to canopy development as was proposed by Inman-Bamber (1991). Senescence could begin when the canopy was only intercepting 50% of incoming radiation. Singels *et al.* (2005a) suggested that competition for light between shoots within the cane stool or crop row is more important than between rows. This would explain the high correlation between bud density and peak stalk population.

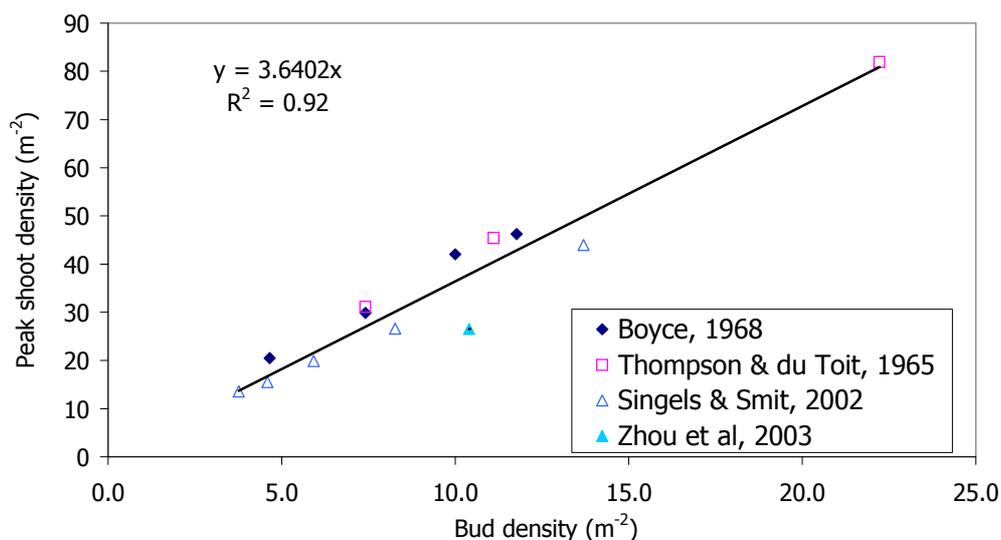


Figure 4. Measured peak shoot density as a function of the initial bud density for various experiments. Bud density was estimated from the amount of seedcane planted (one bud per 10 cm) for Thompson and du Toit (1965) and Singels and Smit (2002). Zhou et al. (2003) and Boyce (1968) measured bud density. Reprinted from *Field Crops Res*, 92, 2-3, Singels *et al.* (2005), with permission from Elsevier.

Restraints on reaching crop potential yields

Flowering

Flowering is desirable as a trait for breeding purposes but is undesirable for production. Flowering can be suppressed in experiments by disrupting the photoperiod (daylength) sensed by plants using a ‘night break’ such as incandescent lighting over the crop for 45 min at midnight. Berding and Hurney (2005) reviewed the work of such experiments conducted in Australia and South Africa and concluded that flowering can reduce sucrose yields by about 10%, enough to warrant selection against flowering in Australian breeding programmes. In South Africa there is no selection pressure against propensity for flowering. It is believed that varieties that flower could be managed agronomically to achieved high yields and avoid negative impact from flowering (Donaldson and Singels, 2004). Breeding with shy-flowering clones has been achieved for many years by controlling the photoperiod in purpose built facilities (Brett, 1974; Berding and Moore, 2001). New evidence presented by Berding and Hurney (2005) suggested that photoperiod, soil water *and* temperature need to be optimum for flower initiation. Daily maximum temperatures >32°C were found to inhibit flower initiation (Berding and Moore, 2001; Berding and Hurney, 2005) even when soil moisture and photoperiod conditions are adequate. In Meringa, Australia, high temperatures can be avoided by delaying the commencement of initiation in photoperiod facilities until 1 April.

Lodging

When cane was held erect with bamboo scaffolding in a series of experiments, sucrose content (or CCS) increased by 3-12%, and sucrose yield by 15-35% (Singh *et al.*, 2002). Berding and Hurney (2005) devoted considerable attention to component traits (stalk height, number, diameter and shear strength) that could be responsible for the unwanted lodging trait. Stalk height was the only component trait that was related to lodging. Early selection of erect clones on the basis of stalk height would guarantee clones with short stalks and probably low yield. Berding and Hurney (2005) suggested that a combination of breeding and management approaches need to be used to reduce yield losses due to lodging. One of the ways in which lodging can affect yield is through the disruption of the canopy and therefore reduced radiation use efficiency, a topic covered in the next section.

Radiation use efficiency

Radiation use efficiency (RUE) of sugarcane, defined as above ground biomass produced per unit of global radiation intercepted, was reviewed and revisited in two papers (Singels *et al.*, 2005b; Park *et al.*, 2005). Singels noted the reasonable agreement in maximum RUE (1.59 to 1.72 g/MJ) reported by various authors. Singels and Smit (2002) found that RUE declined from 1.72 to 1.25 g/MJ, as row spacing for a plant crop of cultivar NCo376 declined from 2.66 to 0.73 m. This interesting observation was attributed to shading of lower leaves by upper leaves where photosynthesis may be reaching maximum rates. Singels *et al.* (2005a) suggested that wider rows would allow more solar radiation to reach lower leaves and allow them to contribute more to canopy photosynthesis. Their data also indicated that RUE may be higher during early rather than late stages of crop development for the same reasons that RUE is greater in wide than in narrow row spacings.

Singels *et al.* (2005b) noted the considerable disparity in the way models deal with the influence of temperature on RUE or net photosynthesis. In some models RUE is constant over a wide range of temperatures, while in others, RUE and photosynthesis are more directly related to temperature. They developed some equations based on published models and found that inclusion of temperature and maintenance respiration improved the simulation of seasonal variation in RUE. However, it was clear that more improvements are required. Models need to account for temperature effects on RUE, preferably through the simulation of growth and maintenance respiration as separate processes from photosynthesis.

Park *et al.* (2005) analysed biomass accumulation and cumulative radiation interception data collected from 14 growth analysis experiments and a total of 34 treatments in Australia. In almost 50% of these treatments, RUE declined significantly before harvesting. A number of causes of this growth decline were investigated. Although the exact time and severity of lodging was not known, notes taken indicated that lodging was at least partly responsible for reduced RUE. However, in some experiments which lodged, RUE did not decline before harvest. In all experiments, specific leaf nitrogen (SLN = N mass per unit leaf area) declined with crop development. By the time approximately 4000 g/m² of biomass had accumulated, SLN in almost half of the treatments fell below 1.2 g/m² which is a threshold for reduced RUE in maize (Sinclair and Horie, 1989). However, no difference in SLN could be found between treatments in which RUE declined and those where it did not. Reduced SLN remains a 'suspect' for RUE decline and needs

to be subject to more critical research. As with SLN, stalk population generally declined over time in all treatments. However RUE was highly correlated with stalk loss after it declined in certain treatments (Figure 5). Stalk death, possibly as a result of lodging, would lead to reduced biomass or even negative accumulation (Figure 5) despite the capacity of remaining stalks to compensate (Bell and Garside, 2005).

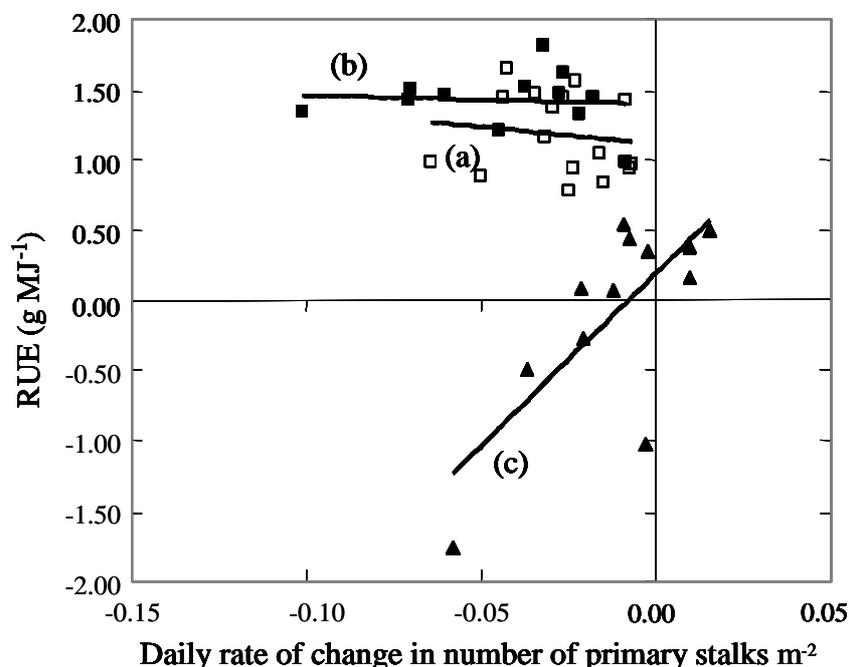


Figure 5. The relationship between the daily change in the number of stalks/m² from the first count taken at approximately 80 days after crop start and (a) maximum RUE in treatments where no RUE decline was observed (□), (b) maximum RUE before it declined (■), and (c) average RUE after the decline (▲). Linear regression for (a) $y = -2.48x + 1.11$, $r^2 = 0.02$ (n.s.), (b) $y = -0.62x + 1.40$, $r^2 = 0.01$ (n.s.), and (c) $y = 24.45x + 0.19$, $r^2 = 0.54$ ($P < 0.01$). Reprinted from *Field Crops Res*, 92, 2-3, Park et al. (2005), with permission from Elsevier.

DM partitioning

Stalk fraction of biomass

A range (0.59 to 0.73) in maximum stalk fraction of biomass (at harvest) was found in the literature (Singels *et al*, 2005b). Stalk fraction is affected by water stress (Inman-Bamber *et al*, 2002), temperature (Singels and Inman-Bamber, 2002) and cultivar (Inman-Bamber *et al*, 2002). Singels *et al*. (2005b) proposed an interesting hypothesis for the observed seasonal variation in stalk fraction at harvest. Stalk fraction for NCo376 was 0.57 in March and 0.78 in June, possibly because of differential temperature sensitivities of the leaf and stalk growth sinks as proposed by Liu and Bull (2001). Singels and Inman-Bamber (2002) surmised that cooler temperatures favoured partitioning away from leaves towards stalks. Their data showed that in water stress-free crops, differences in partitioning fractions could be attributed to differences in mean temperatures experienced by the crops. Base temperatures for leaf

and shoot emergence (10°C and 16°C respectively, Inman-Bamber, 1991) are not consistent with this hypothesis. However, leaf expansion could be less sensitive to temperature fluctuation than stalk elongation above these base temperatures. It is interesting that stalk elongation is more sensitive to water stress than leaf elongation (Batchelor *et al.*, 1992; Inman-Bamber 2004).

Sucrose fraction of stalk dry mass

Singels reviewed research on developmental and seasonal variation in stalk sucrose content (S). Many attempts to account for climatic and physiological influences on S have not yet provided a robust model of sucrose accumulation at the whole stalk level. In a new stalk model, Singels and Bezuidenhout (2002) divided the stalk, length wise, into mature and maturing sections. In their model, whole stalk S depends on the mass and S of stalk which is mature and the mass and S gradient of the remaining stalk. The S gradient of the immature section depends on source-sink responses to water, nitrogen, temperature and radiation. All of these probably interact with genotype. Singels *et al.* (2005b) demonstrated the influence of temperature on this gradient for NCo376 and for two hypothetical cultivars, H3 and H4 (Figure 6). Cultivar H3 partitions more assimilate to sucrose and less to stalk structure at any temperature compared to NCo376, while cultivar H4 partitions more sucrose at high temperatures and slightly less at low temperatures. Partitioning to sucrose in all cultivars is reduced as temperature increases (Figure 6). This model was able to mimic the distinctly different seasonal patterns of S at harvest, of early season and mid season varieties.

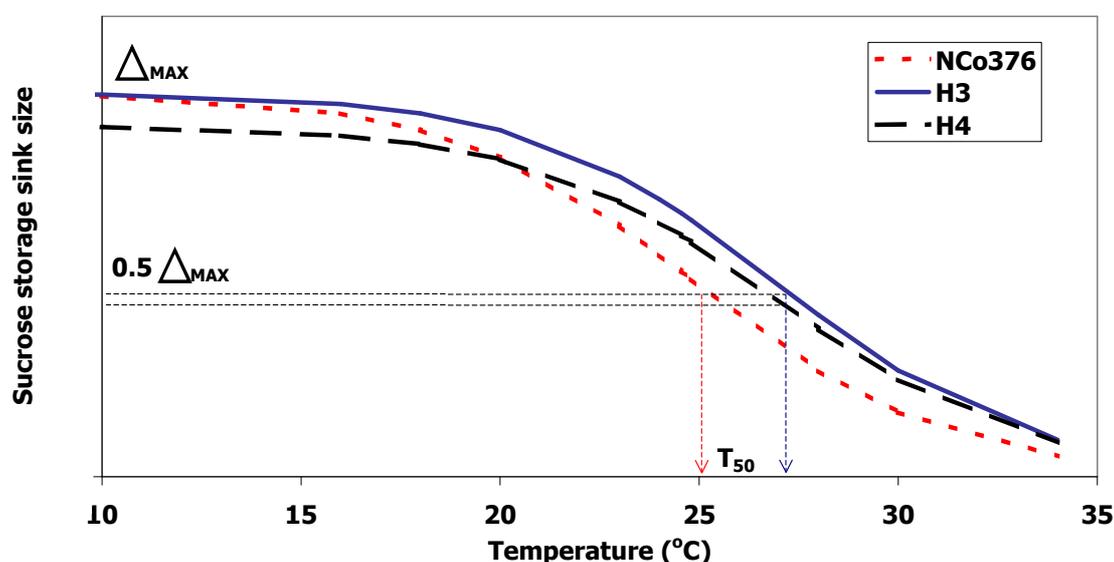


Figure 6. The effect of temperature on the capacity of three cultivars to store sucrose in the stalk. These functions are determined by the parameters Δ_{MAX} and T_{50} . Reprinted from *Field Crops Res*, 92, 2-3, Singels *et al.* (2005b), with permission from Elsevier.

This approach also provides a good framework for linking up with enzyme studies on sucrose metabolism (Ebrahim *et al.*, 1998). Levels of enzymes such as acid invertase and neutral invertase are strongly correlated with sucrose levels and growth rates in different parts of the stalk (Hatch and Glasziou, 1963). Both Hatch and Glasziou

(1963) and Ebrahim *et al.* (1998) have found very close correlations between acid invertase activity in immature stalks and temperature. Understanding the processes within immature and ripening internodes at the cellular level, as being investigated by Rae *et al.* (2005) will add another dimension to understanding and manipulating the proportion of mature internodes.

This approach to simulating genotypic and environmental control of assimilate partitioning to sucrose seems promising and further refinement of models could realize their potential for assisting crop improvement and management. An accurate model could for example be used to identify desirable traits (or combinations of traits) for specific agro-climatic situations and point the way to appropriate selection criteria. Another potential application is to identify appropriate agro-climatic environments for selection trials as suggested by Jackson and Galvez (1996). Crop management could be supported for example, by applying an accurate model and weather data to identify cultivar x age x soil combinations that are likely to respond sufficiently to chemical ripener treatment, given recent and expected future weather conditions, to produce worthwhile economic returns.

Breeding

Jackson (2005) provided convincing evidence for the lack of success in improving CCS with conventional breeding in Australia over many years. His remedy for this situation included:

- A 'high CCS' breeding programme based on recurrent selection and short generation intervals where selection is weighted heavily on CCS.
- Investigation of the physiology of improved CCS including genetic correlations between sucrose content and cane yield to assess whether there are problematic trade-offs between assimilate partitioning and growth at high CCS levels.
- Basic research using parental clones and progeny currently under use and evaluation in breeding programmes to provide precise estimates of the key genetic parameters of additive genetic variance, narrow sense heritability, and genetic correlations among traits, for cane yield, CCS and other economically important traits. From these parameters and statistical theory, optimal selection indices for maximising rates of genetic gain for economic value for use in modern breeding programmes may be determined.
- Conducting studies in progeny populations derived from exotic germplasm, including quantitative trait loci (QTL) mapping in populations derived from backcrossing this germplasm into elite, high CCS, breeding programme parents, to identify whether this approach could be used to identify and introgress new genes contributing to higher sucrose content in current parental material and cultivars.

Nutrition

Sugarcane nutrition received comparatively little attention at the workshop. Thorburn *et al.* (2005) described how previous advances in nitrogen nutrition had been incorporated into simulation models of sugarcane, and showed how these models were being used to analyse the interactions between nitrogen fertiliser management and various issues, such as environmental sustainability or trash management. Understanding these issues requires as much knowledge about soil processes as crop

physiology, and many of the recent knowledge advances have been made in the soil domain (e.g. trash decomposition). Equally, many of the unresolved issues, e.g. greenhouse gas emissions, are also in the soil domain. However, knowledge of sugarcane root system function is an important limitation, as are the effects of nitrogen on vegetative growth, including suckering, as opposed to maturation and sucrose accumulation.

Wood and Schroeder (2004) described how off-take of potassium from sugarcane fields in Australia was greater than that generally applied in fertiliser. They questioned the long-term implications of this net removal of potassium from the soil. Potassium is important for osmoregulation, stomatal control and therefore for water stress tolerance in sugarcane. Water stress is common in many sugarcane production areas and cultivar, climate and potassium nutrition interactions need to be investigated and exploited.

Discussion

Space does not allow adequate consideration of the many excellent papers arising from the physiology workshop, however, the summary presented above gives sufficient background to the final recommendations adopted. A more complete record of points raised during discussion and breakout sessions (obtainable from SRDC¹) reveals the intensity of debate around several issues notably suggestions for improving CCS. The workshop identified sucrose storage as the most important process for future research. Water and N use efficiency were also given high priority. Genetic and environmental controls of these processes were highlighted as priorities for future research. Physiological and morphological traits responsible for improved yield, sucrose content and resource use are poorly understood in sugarcane. Delegates reached a consensus that effort should be directed at a better understanding of traits responsible for high yield and high sucrose content and high water and N use efficiency, in order to better design future genotypes.

The work reviewed and presented by Casu *et al.* (2005), Watt *et al.* (2005), Rae *et al.* (2005) and Singels *et al.* (2005b) presents an opportunity to link modelling of whole crop behaviour with gene expression and cellular studies and thus span the range of organisational levels from molecule to crop. Experiments designed to provide coefficients for the partitioning model of Singels and Bezuidenhout (2002) could also provide tissue for micro-array analysis, so that genes responsive to up- and down regulation by a range of well defined experimental conditions (water, radiation, temperature) can be identified. This could provide a clearer picture of how gene expression for sucrose accumulation is regulated by developmental and environmental conditions than has been achieved so far with the limited range of developmental stages considered by Casu *et al.* (2005) and Watt *et al.* (2005). Molecular biologists, plant and crop physiologists, breeders and modellers all need to be involved in this type of research which is a step towards the holistic, top-down/bottom-up approach envisioned by Moore (2005). Sugarcane simulation models are not yet capable of simulating genotype x environment interactions. Research proposed by Singels *et al.* (2005b) to refine their sucrose partitioning model and develop coefficients to describe

¹CSE006 Final report. Sugar Research and Development Corporation, PO Box 12050, George Street, Brisbane, Qld 4003 Australia

how a range of genotypes respond to 'ripening' stimuli, would help greatly in developing capability to model G x E interactions. An accurate model with this capability could be used to identify desirable traits (or combinations of traits) for specific agro-climatic situations and point the way to appropriate selection criteria for sucrose accumulation. Another potential application is to identify appropriate agro-climatic environments for selection trials as suggested by Jackson and Galvez (1996). An accurate model sensitive to G x E interactions could be used to design better farming systems to exploit the wide range of genotypes and management options available to growers.

The sparse literature on the genotype x water relations interaction indicates that there is a wealth of genetic variability in crop response to water deficits. It is surprising that little effort has been devoted to exploring the opportunities that have been presented in the few but significant publications. In Australia drought causes considerable economic hardship to cane growers quite often and yet there is no selection site for drought tolerant varieties. Research into traits conferring drought resistance and increased WUE is warranted. Smith *et al.* (2005) and Bell and Garside (2005) raised the issue of root growth and RUE. It is possible that selection for high yield under generally favourable conditions of breeding stations has led to reduced root vigour. There is a need to evaluate clones and cultivars for response to water deficits to help predict those which may be susceptible to drought and to improve our capability to model the G x E interaction. Smith *et al.* (2005) make a useful suggestion that radiation use efficiency and water and nutrient utilisation should be compared in old and new cultivars to determine if our selection programmes are leading away from efficient resource use in sugarcane farming systems.

Innovative concepts by Singels *et al.* (2005b) for improving sugarcane simulation models need to be developed into algorithms, followed by verification and validation procedures. While all the suggestions are worthy of consideration, the most important are (i) that the size of a leaf depends on source strength of older leaves during the thermal-time opportunity for its development, (ii) photosynthesis and maintenance respiration are temperature dependent, and (iii) sucrose content is subject to a ripening gradient in the maturing stalk and this gradient is subject to genotype x temperature x water stress x ripener interactions.

Apart from the various research issues identified for priority, the workshop voted overwhelmingly for collaboration between scientists working at all levels of organisation in the sugar supply chain. Edmeades *et al.* (2004) have recently identified some of the issues and changes needed to make such collaborations possible. They urge physiologists to adapt methodologies to determine variation in hundreds rather than the few genotypes they are used to studying in detail. They ask geneticists to work with the physiologists in developing genotypes and populations that are more amenable to answer the questions being addressed.

Acknowledgements

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authors of that meeting are thanked for their contributions, which led to both a special edition of *Field Crops Research* and this summary paper.

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Appendix 13
Knowledge, Attitudes, Skills and Aspirations of key members of the BSES-CSIRO-CSR Sugarcane Variety Improvement Program
by Sarah Park

A questionnaire was devised with the help of Drs Mac Hogarth and Emma Jakku (social scientist) to document the Knowledge, Attitudes, Skills and Aspirations (KASA) of key plant breeders working in the BSES-CSIRO-CSR Sugarcane Variety Improvement Program. The questionnaire was completed by 11 people employed presently, or in the recent past, in the Australian breeding program. Nine of the questionnaire respondents were directly involved in daily plant breeding activities.

The aim of the questionnaire was to document the baseline KASA of people associated with breeding at the outset of Project CSE014, 'Increased CCS, cane yield and water use efficiency by exploiting interactions between genetics and management'. The questionnaire will be conducted again in 2009 prior to the end of the project to ascertain if there has been any changes to the KASA of breeders employed in the BSES-CSIRO-CSR Sugarcane Variety Improvement Program.

Selected respondents were guided through the questions during semi-structured interviews conducted by Drs Sarah Park and Geoff Inman-Bamber. The questions were devised to elicit breeder's KASA in regard to incorporating physiological understanding into breeding procedures. Physiological understanding was taken to be a mechanistic knowledge of how the plant works, for example to store sucrose instead of using it for growth. The questions firstly used a simple rating system to capture answers. More open-ended questions were then used to elicit greater understanding of the responses. The interviews were recorded to provide an accurate record of the discussion to serve as the basis for summary and analysis. The recordings and their transcriptions are to remain completely confidential and the respondents are not personally identified. The insights gained during the research are documented below.

The 25 questions were grouped into five categories:

- Choosing the traits for selection
- Use of mechanistic crop models
- Defining the success of a breeding program
- GxE interaction
- Future prospects

Summary of conclusions

- **The BSES-CSIRO Sugarcane Variety Improvement Program generally selects for cane yield and CCS, disease resistance and to a lesser extent some other traits of economic importance. The decision to select for these traits is primarily made by the Breeding Team.**
- **Physiological knowledge presently contributes very little to decisions made regarding trait selection, largely due to the lack of physiological knowledge on sugarcane. Use of physiological knowledge in sugarcane breeding is expected to increase in the next 10 years.**
- **Although there is little or no use of physiological models, in particular APSIM, in the BSES-CSIRO breeding program at present, there is a clear intention to increase their use in the breeding of sugarcane in future years, despite some concerns about model capability.**

- Many cultivars have been released from the variety improvement program in the past 10 years with differing levels of commercial success. The average time taken from seedling to the release of a new cultivar is generally 12 years, but this is expected to reduce over the next ten years.
- The environment is thought to have at least a minimal influence on the expression of a trait. The effect of a grower's management on a cultivar's performance was generally thought to be relatively greater than the management effect occurring during on-station trials.
- Limited information is provided to growers at the time of cultivar release and growers are encouraged to perform their own strip trials to gain more extensive information.
- There were mixed aspirations for a potential increase in cane yield and CCS in the next 10 years. The biggest gains are thought to be achievable in the yield of ratoon crops and early-season CCS.
- Most respondents considered the impact of Project CSE014 on the BSES-CSIRO Sugarcane Variety Program to be at least moderate.

Choosing the traits for selection

All regions have selected for cane yield and CCS over the past 10 years. In addition, many of the regions have selected for early CCS, sugar quality (as measured by floc) and the quality and quantity of fibre content. Agronomic type (or ideotype) has also been selected for in terms of erectness, reduced lodging, reduced suckering and ratoonability. Northern regions have also selected for reduced flowering propensity. Disease resistance is selected for throughout the entire breeding program, with the suite of diseases targeted being region-specific and in accordance with local prevalence and susceptibility.

The choice of trait selected for is primarily driven by economic importance and the dollar return to the industry. By far the greatest contribution to the decision-making process is from the Breeding Team (Fig. 1), but the opinions of local industry (growers and millers) are canvassed, either formally or informally. In one region local extension staff are incorporated into the decision-making process and to a lesser extent pathologists are also involved in deciding which traits will be selected for.

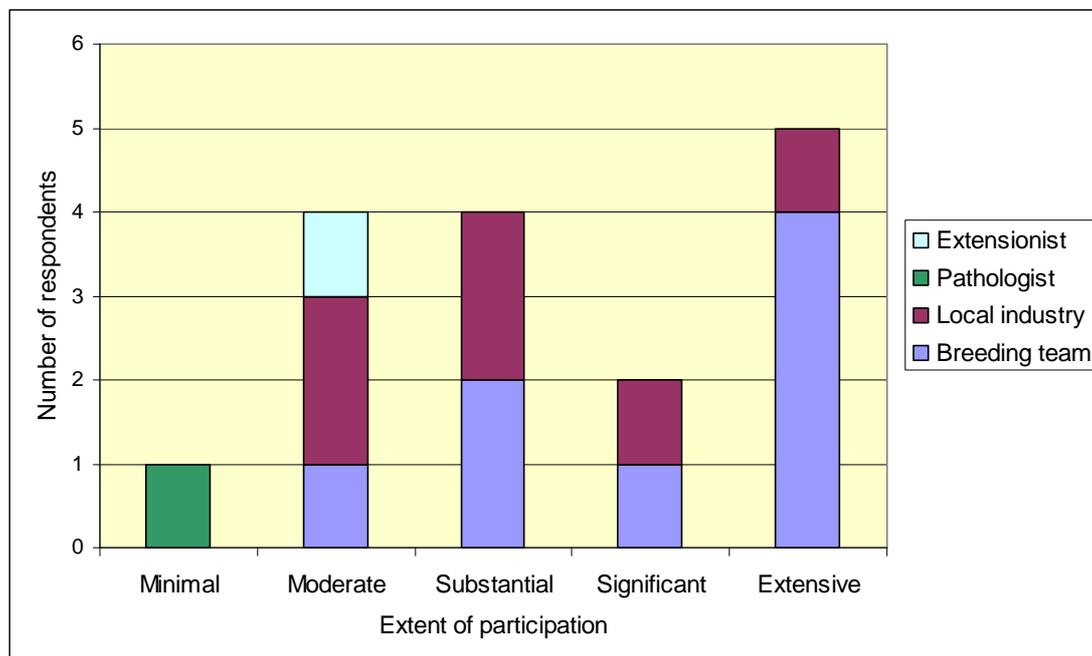


Figure 1. The level of participation in the decision-making process.

The respondents were questioned as to the extent to which knowledge of plant physiology contributed to their choice of traits selected for in the present breeding programs in the region. The majority of respondents considered this to be either none or minimal, although 4 respondents thought that physiology contributed at least moderately to their decision on what traits to select for (Fig. 2). In general, a lack of physiological knowledge was cited as the main reason for not incorporating physiology into the decision making process. When asked at a later stage in the interview about the extent of their physiological knowledge, respondents generally considered themselves to have a moderate level of knowledge (Fig. 3). Around 65% of respondents knew of the use of physiological knowledge in breeding trials for other crops, e.g. wheat, corn, rice, sorghum. These respondents generally rated the use of this knowledge to have provided moderate to significant benefits to the breeding program.

Where physiological knowledge has been incorporated into decision-making for trait selection in sugarcane, this has generally resulted in the decision to select for erectness. All respondents were optimistic about an increase in the use of physiological knowledge in sugarcane breeding

over the next 10 years (Fig. 2). It was thought that the benefits would be realised through a better physiological understanding of what is limiting crop growth, an improved focus on desirable traits, the ability to interrogate past breeding records to understand what traits have provided increased productivity and the development of new selection criteria for identifying traits. Water use efficiency, drought tolerance, lodging, suckering and nitrogen use efficiency are all seen as traits likely to be identified as having potential for improving crop performance. It was thought that these benefits would have the potential to reduce inputs and the time from crossing to cultivar release. The constraints likely to scupper these benefits from being realised are thought to include insufficient financial resources to enable both an increase in physiological knowledge and the time for the respondents to acquire it, the inherent risk involved with taking a new approach, the concomitant education of local industry and a realisation of the mutual desire for physiologists and the breeding team to collaborate effectively. These constraints were viewed as anything from minimal to extensive (Fig. 4). There was a general consensus that there were likely to be no shortcomings involved in incorporating physiological knowledge into the breeding process, with the exception of one respondent who expressed concern about the inherent risk of adopting new approaches.

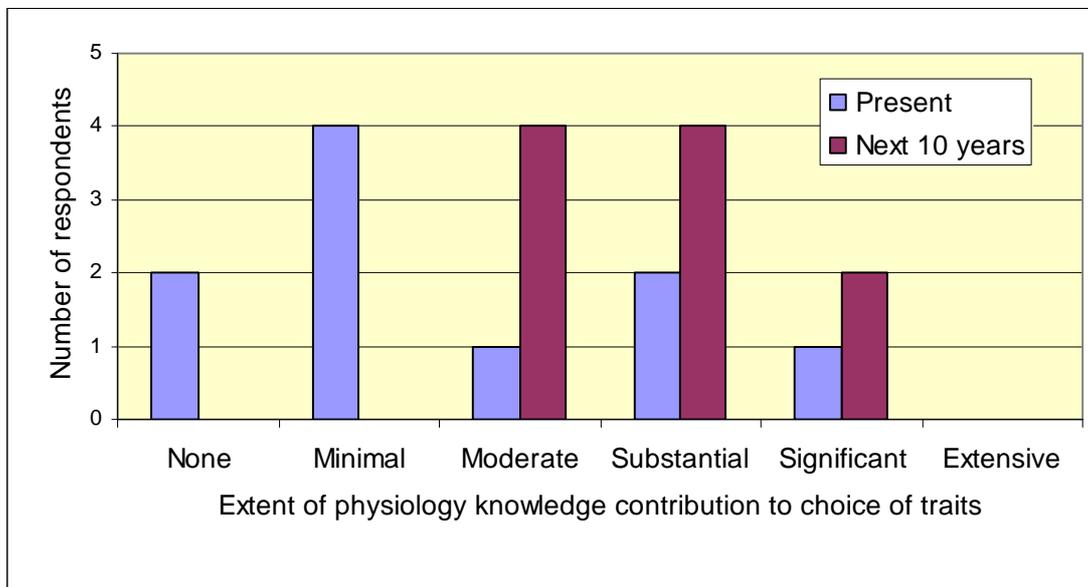


Figure 2. Extent to which knowledge of physiology has contributed to the selection of traits in the present breeding program and its potential to be incorporated into decision making in breeding programs in the next 10 years.

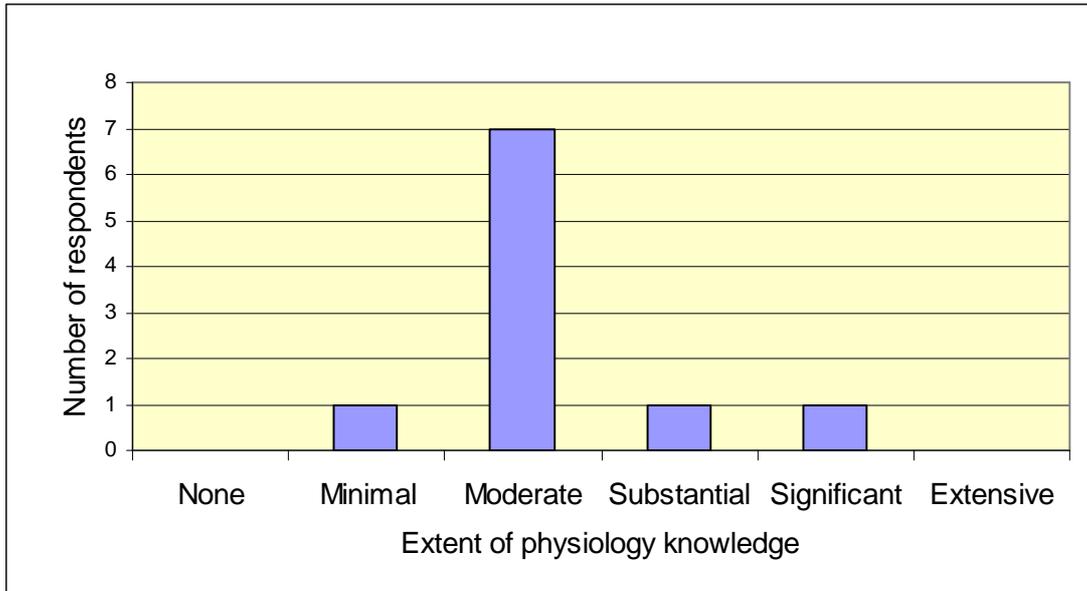


Figure 3. Extent of physiological knowledge of respondents.

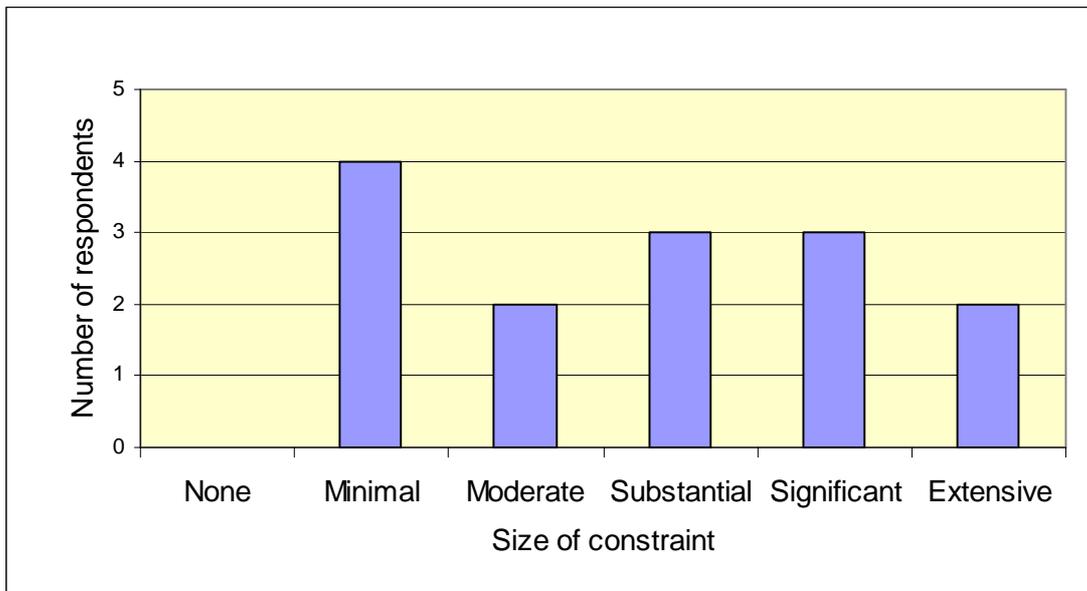


Figure 4. Size of the likely constraints prohibiting greater incorporation of physiological knowledge into the breeding program.

Conclusion

- The BSES-CSIRO Sugarcane Variety Improvement Program generally selects for cane yield and CCS, disease resistance and to a lesser extent some other traits of economic importance. The decision to select for these traits is primarily made by the Breeding Team.
- Physiological knowledge presently contributes very little to decisions made regarding trait selection, largely due to the lack of physiological knowledge on sugarcane. Use of physiological knowledge in sugarcane breeding is expected to increase in the next 10 years.

Use of mechanistic crop models

There was a clear indication that mechanistic/physiological models, such as APSIM, were currently not being used to any great extent in the breeding procedures in the Australian sugar industry (Fig. 5). Respondents displayed knowledge of their use in breeding trials for other crops, e.g. wheat, barley and sorghum. However, there was a range in the level of aspiration regarding their potential to provide a benefit to the sugarcane breeding program. This ranged from being able to provide a substantial contribution, to failing to contribute in any way. It was interesting to note that when respondents were asked to estimate the extent to which these models, and in particular APSIM, would contribute to improvements in the BSES/CSIRO breeding program in the next 10 years, all respondents considered that they would provide at least a minimal contribution, with some respondents considering the extent of the contribution would be substantial. A possible explanation for the range in responses may be concerns expressed about potential constraints to the implementation of models. These included the difficulty in modelling genetic differences between cultivars when these were likely to be small in comparison with relatively larger environmental effects.

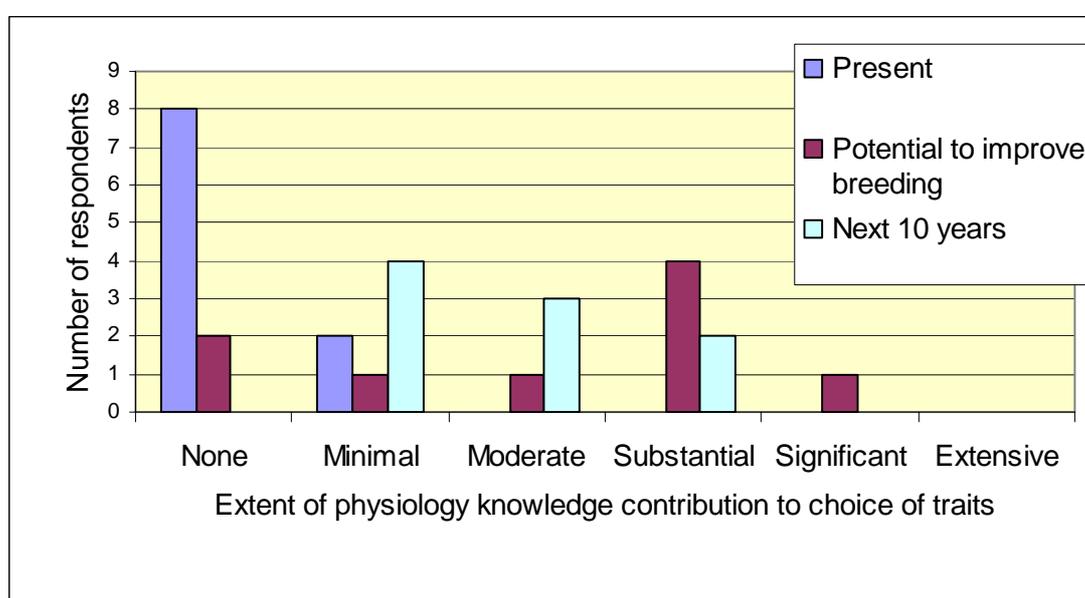


Figure 5. Extent to which mechanistic/physiology contribute to the BSES-CSIRO Sugarcane Variety Improvement Program at present and in the next 10 years, and their potential to improve the breeding process.

Conclusion

- Although there is little or no use of physiological models, in particular APSIM, in the BSES-CSIRO breeding program at present, there is a clear intention to increase their use in the breeding of sugarcane in future years, despite some concerns about model capability.

Defining the success of a breeding program

Many cultivars have been released from the BSES and CSR sugarcane variety improvement programs in the past 10 years. These have had differing levels of commercial success. The level of success of a commercially released cultivar is measured using a range of direct and indirect variables. The direct measure of economic impact of the cultivar (as calculated using area of land, relative increase in yield or percentage of mill supply) is widely considered the benchmark for evaluating success. More indirect measures include an estimate of genetic gain,

an assessment of whether the industry is still planting the cultivar after 3 or 4 years of its release, feedback from growers and the rate of adoption. The latter was considered to be a poor indication of success by one respondent as it was thought that this proxy measure does not take into account those growers who are trying out a new cultivar, but fail to continue planting it. The high level of success achieved in the past has been credited to achieving improvements in the main traits being selected for, the good collaborative relationship between the breeding program and the local industries and the rigorous nature of the breeding procedure used.

The average time taken from seedling to the release of a new cultivar is generally 12 years, though punts on some clones has resulted in the release of cultivars in only 8 years. The need to transport clones through inter-station exchange and quarantine can increase this duration. However, one respondent considered that prevarication amongst breeders and the institutions involved has resulted in the release taking anything up to 21 years. Around 70% of respondents thought that the average time to release could be reduced through a combination of a reduction in the number of selection stages and the introduction of molecular marker technology. The minimum time for release of a cultivar was thought to be 6 years. Reasons given by respondents who indicated that it was either not desirable or possible to reduce the release time, included an undesirable reduction in observational data associated with the increasing use of mechanical harvesters and concerns about releasing cultivars with unknown undesirable traits.

Conclusion

- **Many cultivars have been released from the sugarcane variety improvement program in the past 10 years with differing levels of commercial success. The average time taken from seedling to the release of a new cultivar is generally 12 years, but this is expected to reduce over the next ten years.**

GxE interaction

There was a range of views expressed about the size of influence that the management of selection trials sited on the experiment stations had on the clones selected, in particular the management of fertilizer application rates, irrigation scheduling, row spacing and number of rows per plot (Fig. 6). These ranged from none to substantial across the regions. All respondents said that they adopted the same practices as growers in the region with the exception of the number of crop rows planted. It was well recognised amongst the respondents that the competition effect resulting from a single row plot greatly influenced cane yield, but had a minimal effect on CCS. Whilst one respondent stated that the current practice is to assume no effect of environment or management, he stated that this might not be a correct assumption and it will require better methods of analysing and interpreting data before this is known.

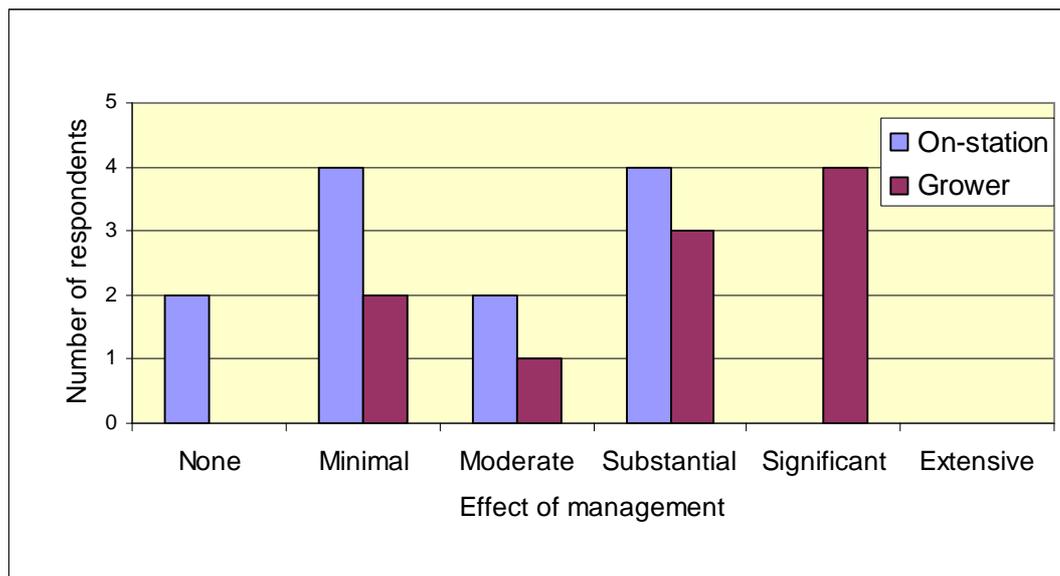


Figure 6. Extent of influence of management of selection trials on the clones selected by breeders.

Respondents considered that the effect of a grower's management on a cultivar's performance was, in general, relatively greater than the management effect occurring during on-station trials (Fig. 6). Interestingly, breeders considered that the amount of information they supplied to growers at the time of cultivar release was generally very limited, but it was evident that there was a large disparity across the breeding regions as to how much information was released with the cultivar. This information might include recommendations on a number of the following agronomic factors: the time of harvest, soil types, herbicide and disease tolerance, germination, ratooning ability, nutrient requirements, planting window, tolerance to waterlogging, drought and frost and abundance of flowering. However, more than one respondent considered that as the information was generally based on a small number of trials, its reliability was likely to be questionable.. In all regions, growers are encouraged to perform their own strip trials to gain more extensive information.

Respondents were mixed in their thoughts about the extent to which the specific soil and climate conditions in their region have influenced the expression of a trait in a cultivar that has been released by the BSES-CSIRO Sugarcane Variety Program (Fig. 7). Interestingly, all respondents considered that the environment had at least a minimal influence on the expression of a trait.

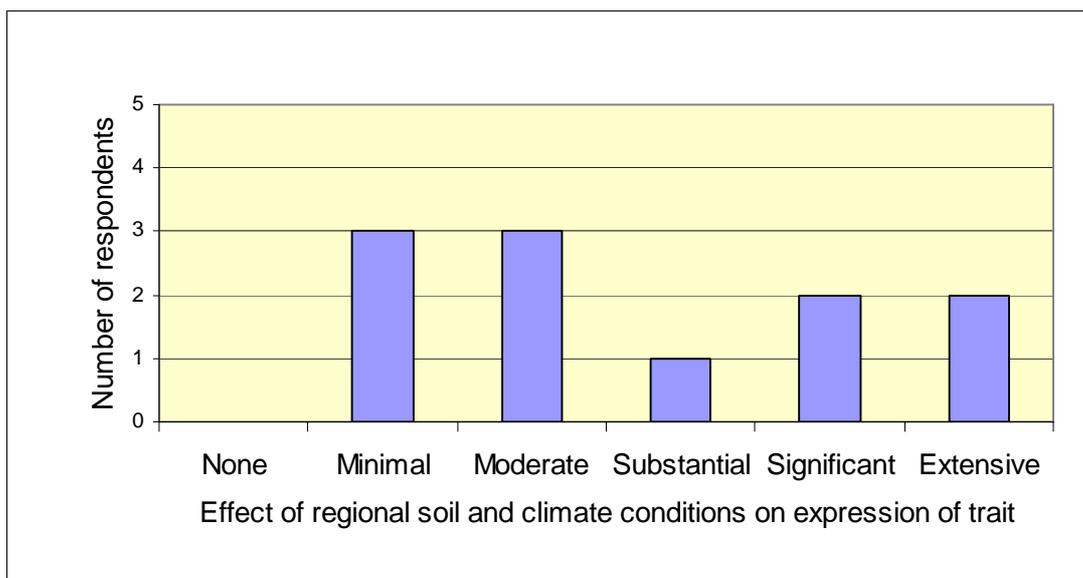


Figure 7. The size of influence that the management of selection trials sited on the experiment stations had on the clones selected.

Conclusion

- **The environment is thought to have at least a minimal influence on the expression of a trait. The effect of a grower’s management on a cultivar’s performance was generally thought to be relatively greater than the management effect occurring during on-station trials.**
- **Limited information is provided to growers at the time of cultivar release and growers are encouraged to perform their own strip trials to gain more extensive information.**

Future prospects

Respondents showed mixed aspirations for a potential increase in cane yield and CCS resulting from the BSES-CSIRO Sugarcane Variety Program in the next 10 years (Fig. 8). In terms of cane yield the biggest gains are thought to be achievable in ratoon crops, more than in plant crops. Greater knowledge of lodging and suckering and how it impacts final yield is also thought to lead to these gains, as is the capture of specific combining ability with proven crosses. It was also considered that getting high yield is relatively easy, but trying to combine it with disease resistance and high CCS is the greater challenge. Those respondents considering that gains in cane yield were likely to be minimal, cited previous gains achieved and questioned if any future increases in cane yield potential were possible.

It is generally considered more difficult to make gains in CCS, but 3 respondents thought that the potential for this was either substantial or extensive (Fig. 8). Two respondents thought that gains in CCS were more likely to come from increasing CCS early in the milling season because ‘early’ CCS has not been selected for in the past and there is genetic variation available for this trait. Those respondents considering that gains in CCS were likely to be minimal cited the high cost of the breeding program and its present labour-intensive practices prohibiting further progress.

Constraints thought to potentially restrict increases in cane yield and CCS included the current threats to BSES’s existence and limited financial resources (particularly in terms of its limitations on the annual number of crosses that can be made).

Conclusion

- There were mixed aspirations for a potential increase in cane yield and CCS in the next 10 years. The biggest gains are thought to be achievable in the yield of ratoon crops and early-season CCS.

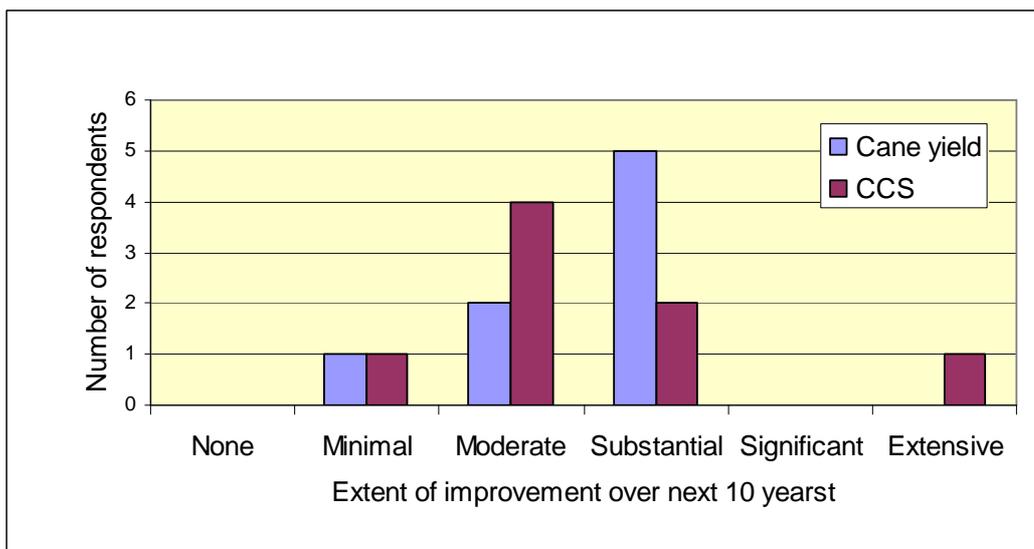


Figure 8. Potential increase in cane yield and CCS resulting from the BSES-CSIRO Sugarcane Variety Program in the next 10 years.

Box 1 contains the research aims for Project CSE014, 'Increased CCS, cane yield and water use efficiency by exploiting interactions between genetics and management'. Respondents were asked their thoughts on how the aims of the project would impact on the breeding program in their region.

Box 1. Aims for Project CSE014.

- To better understand the interactions between sugarcane genetics and environment (including management) with respect to sugar accumulation and lodging.
- To improve variety choice and cane management by growers for increased CCS and cane yield.
- To determine improved selection systems to identify elite varieties with desired characteristics.
- To develop novel irrigation strategies to prevent lodging and maximise CCS.
- To promote better management practices for maximising sugar yield and reducing water use.
- To link studies at a molecular (gene) level to traits measured at the crop and plant level to increase mechanistic understanding and explore potential for use in future selection.

The two respondents who considered that Project CSE014 would have none and minimal impact on the breeding program in their region responded in terms of the 4-year life of the project (Fig. 9). Both respondents were more optimistic about positive benefits that may results from a more long-term approach. Without specifying the time duration, around 65% of all respondents considered the impact of the project to be at least moderate.

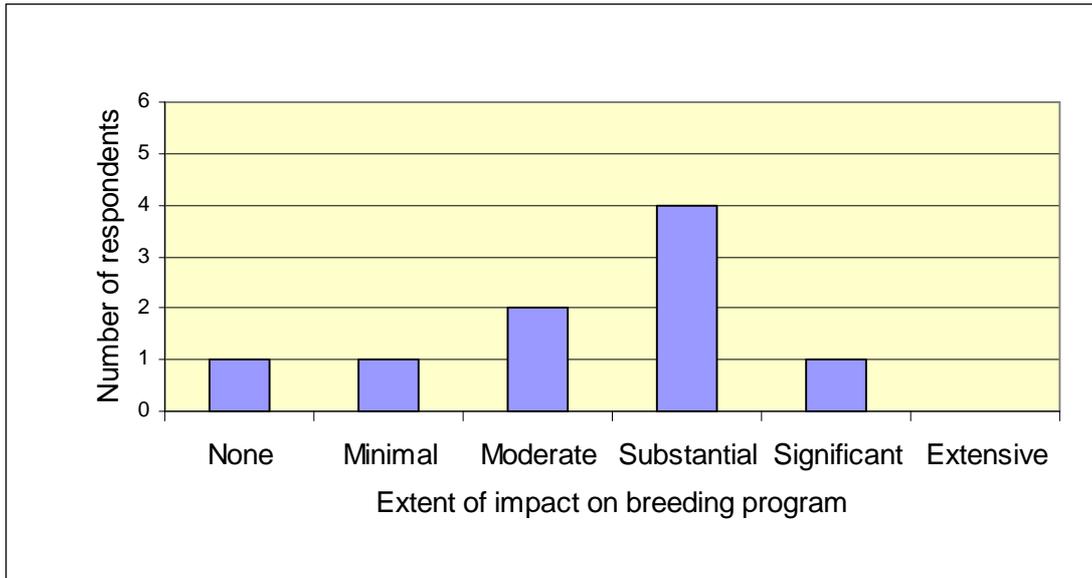


Figure 9. Extent of impact of project aims on breeding program.

Conclusion

- **Most respondents considered the impact of Project CSE014 on the BSES-CSIRO Sugarcane Variety Program to be at least moderate.**

Appendix 14
Progressive evaluation project CSE014

	Date	Place	Event
1	10 Aug. 05	Townsville	Workshop and steering panel
2	17 Oct. 06	Townsville	Workshop and steering panel
3	16 Oct. 07	Townsville	Workshop and steering panel
4	1 Oct. 08	-	Milestone 8. Interim evaluation of sub-traits
5	7 Oct. 08	Brisbane	Mini-workshop on sucrose accumulation
6	26-27 Nov. 2008	Townsville	Workshop and steering panel
7	5 Mar. 09	Durban	Mini-workshop on molecular to whole plant knowledge of sucrose accumulation
8	1 April 09	-	Milestone 9. Contribution of project to breeding technology evaluated against baseline

1. Notes from a workshop to finalize the experimental approach to be taken in the project – 10 Aug. 05.

A workshop with the steering panel and the project team was held at Davies Lab, Townsville on 10 August 2005. Those present were: Graham Bonnett, Alan Garside, Phil Jackson, Scott Chapman, Andrew Barfield, Ian Haigh, Sarah Park, Ian Biggs, Nils Berding, Andrew Lashmar, Andrew Wood, Geoff Bamber and Rob Troedson.

Possible approaches for linking biotech, breeding and physiology and agronomy (G Inman-Bamber).

The project team and SRDC have chosen a GxE physiology (gene expression) approach focusing on sucrose accumulation and lodging traits. Other approaches considered were, to investigate traits responsible for variety improvement and variation in traits amongst current varieties. Yet another approach is to consider genes (QTLs) associated with 'desirable' traits. This is the current Sth. African approach which is potentially risky if the trait selected has many genetic controls. The general consensus of the project preparation workshop was to support the approach as developed in the proposal.

Project methodology (G Inman-Bamber).

Two traits will be studied; CCS and lodging. To enhance CCS we plan to control translocation of sugars to the stalk. We can slow down expansive growth by mild water stress in order to redirect sugars to storage. We will aim to irrigate so that plants are in the region where plant extension is reducing but photosynthesis has not started to decline. We have shown in previous research that lodging is linked to the amount of irrigation water applied, more water – increased lodging. There is potential then to regulate the application of irrigation and so delay lodging. Plant height and hence lodging will be restricted before the wet season by reducing irrigation and rapid growth will be encouraged after the wet season. Varieties that are prone to lodging will be compared to those that are not, in a manner similar to the CCS experiment.

What can the understanding of the mechanisms and genetic variation at a molecular level contribute to the project (understanding sugarcane)? What can a reductionist contribute? (G Bonnett)

The CCS trait can be dissected in many ways. In one approach component traits can be those of sucrose, hexoses, fibre and water. Another way is look at plant parts such as the stalk as a line of internodes at different stages of development. Basal internodes are the most mature and

have highest CCS, while younger internodes have less CCS. We can view CCS at the level of 'metabolomics' which is the association of metabolites and development with trait of interest (CCS). We find the certain groups of metabolites increase, while others decrease as you move down the stem from leaves, to immature stem tissue, to mature stem tissue. Gene expression is yet another step in complexity. Using chips of known genes we can look at changes in expression for a variety of tissues or tissues from plants subjected to different environments. This is similar to metabolomics, in that changes in expressions are monitored. The chips show what genes are being switched on at the time of measurement. By combining all approaches for different conditions, a study of trait of interest can be conducted, such as, water stress on CCS.

Modelling gene to phenotype relationships in field crops (Scott Chapman).

Here we want to reduce complexity by identifying underlying genetic basis. An example comes from sorghum research, where David Jordan (QDPI&F) was trying to find basis for "stay green" effect (green leaves at the end of season). Can we understand the interactions that result in "stay green" effect? Modelling gene to crop includes the traditional dogma of increasing layers of detail in the model. Here we aim to identify key linkages across levels of organisation (layers) to build models that explain the processes. For example, in sorghum, flowering is an important stage. Leaves sense the environment. If the environment changes, the leaves detect this and change the phenotype and performance. The response is at a different scale to the sensing, e.g., a flowering response to a leaf environmental sense.

What can breeders benefit from and contribute to the project? (Phil Jackson)

Breeders want a faster way to improve productivity through varieties than current methods. This includes production of varieties that are better suited existing production environments or to some new production environment, whereby the combination of variety x management may have some synergistic effect not achieved by manipulation of either one alone. As a breeder, what I hope we get from this project is:

- Better knowledge of the major biological (environmental, physiological, genetic) constraints to higher CCS and cane yield in our current varieties, especially CCS.
- A good connection between the commercial crop level, the whole plant level, the tissue level, and sub-cellular physiology level, and DNA level of genetic variation in CCS in responding to environmental conditions. Then arising from this, how to productively manipulate the plant at a DNA level (either employing marker assistance, MAS, in combination with traditional crossing and selection, or transgenic approaches.)

Row spacing x variety trial at Gordonvale (Alan Garside)

Controlled traffic requires at least 1.8 m row spacing. A hypothesis proposal is that varieties that sprawl (not lodge!!) would better use the available space between rows. Initial results from trials testing this hypothesis suggest that there are some significant varietal differences in response to farming systems. [Row spacing will not be investigated in the new physiology project but trials will be managed on the best advice out of the YDJV including the use of 1.8 m row]

What can growers benefit from and contribute to the project? (Andrew Barfield)

Grower's main interest is gross margins per hectare; increasing productivity and reducing costs. Our interest in the project then is for increased productivity through improved genetics and/or improved management. This is a strategic project and leads to practical outcomes are yet to be developed. The project could lead to better utilisation of resources especially in

systems such as the Burdekin. Growers will co-operate by reducing barriers to adoption. We can envisage this in taking on changed irrigation strategies. I suggest that you select good growers [to try new technology] and others might pick and adopt the new methods.

What can millers benefit from and contribute to the project? (Andrew Wood)

It is difficult to see what millers will get from, and what they can provide to the project. However important issues for millers are: 1) fibre % and quality, 2) crop size, 3) constraints to cane yield – stalk death at lodging 4) high CCS and a need to even out CCS supply to the mill through the season 5) harvesting – fibre quality issues in the mill also difficult to harvest, 6) cane billet quality and extraneous matter requires increased management in mills, 7) fibre % and quality, 8) sugar quality, 9) season length. Millers can act as industry contacts and be available for discussion about the project.

Are any changes required to project objectives? (Rob Troedson).

Project aims are very ambitious and we need to keep focus on varieties, CCS and lodging. No changes to the objectives were suggested.

Are any changes required to project methodologies or evaluation process? (Robert Troedson)

Ian Haigh: We must go to “new” a way of farming as shown by YDJV using 1.8 m row spacing, permanent beds and trash blanketing. Work up beds, plant a soybean crop and leave fallow over the wet season for an April plant. Keep management as “state-of-art” as possible.

Cultivar selection:

GBonnett: Suggests using genetic mapping population which has similar genetic parents and good CCS data.

PJackson: It is important to link mapping arrays with physiological experiment on some plants that have data from all levels of physiological measures

Proposed changes to the research plan

A meeting was held on 19 August to follow up recommendations of the panel regarding the use of new farming systems and the bulking up of clones. Those present were Alan Garside, Terry Morgan, Mike Spillman and Geoff Bamber.

Additions to the proposed plan were:

- Bulk up 30 odd clones (September 05 to April 06 in 3 row x 10 m x 2 rep plots) to provide sufficient plant material for six plots for each clone (7 rows x 23 m each plot, about 1000 m total row length per clone). Establish as a trial with Q117 guard on outside rows and trial ends.
- Clones selected for the CCS trial will be different to those selected for the lodging trial, otherwise seed requirement will double. We will obtain CCS accumulation data from the lodging trial as well so using different clones will give us a chance to consider a total 8 clones.
- Plant two reps of each clone in bulking plots which are at least three rows wide.
- Take some basic physiological and morphological measurements on clones in bulking plots such as leaf size, leaf appearance rate, stalk numbers and size, photosynthesis, chlorophyll fluorescence.
- Plant CCS and Lodging trials after soybeans using 1.8 m single rows and minimum tillage.

Deletions from plan were.

- 'Duplicate experiments will also be planted for use as ratoon crops in year 3.'
- 'Experimental sites prepared in year 2 will be machine harvested and weighed. This will permit a comparison of detailed data obtained by careful sampling with harvest data of direct relevance to growers and millers.'

SRDC (Rob Troedsen) agreed to these changes which do not affect milestone achievements at all.

Clones and varieties selected for the project and planted in bulking up plots

On the recommendation of the steering panel and with the help of Phil Jackson, Graham Bonnett and Terry Morgan, 34 clones and varieties were chosen for bulking up. Six commercial varieties were included and the unselected clones came from three mapping (segregating) populations for which genetic markers are already known. These entries included high and low CCS traits. Each entry was planted in two replicate blocks and in plots with three rows, 10 m long and 1.8 m apart. The objective of the propagation trial was not only to bulk up planting material for the 2006/07 experiments but also to determine the range of physiological and morphological traits in the segregating populations and in a selection of commercial varieties. We plan to obtain information on the following traits:

Trait	How and when	Destructive
Tillers/main shoot	Primary shoot/stalk counts before tillering, at peak tillering and after tiller senescence	N
Leaf size	Sample stalks at 8 and 16 leaves	Y
Leaf appearance rate	Count leaves 4 or 5 times	N
Early growth habit	Rate the angle of vertical growth 2 or 3 times	N
Stalk height/mass	At 16 leaves	Y
Photosynthesis (2nd leaf)	At 6 and 10 leaves	N
2nd leaf N content	At 6 and 10 leaves	Y

Irrigation trial planned to test techniques for reducing lodging and maximising CCS

A pilot growth control trial was planned and planted on 13/14 September 2005 at CSR's Kalamia estate. The concept of the experiment is to gain control over plant height through irrigation, reducing plant height by a small amount (10 to 30%) before the wet season in order to reduce lodging or after the wet season to enhance CCS. We will be using auxanometers to measure hourly and daily plant extension and EnviroSCAN soil moisture sensors to determine what soil water extraction patterns are associated with reduced growth. Before the wet season, the 'after wet season' treatment will be irrigated as in one of the 'degree of reduction treatments' possibly 25% because we don't want lodging to complicate measurements after the wet season. Essentially we have two experiments each with 16 plots and each sampled for biomass only once and not necessarily at the same time. However we will need to measure stalk height and CCS periodically but not too often.

2. Notes from workshop on 17 Oct. 06.

The following plan was suggested and discussed for glasshouse work on sucrose accumulation.

The panel had previously agreed to support a switch of effort from the field to the Tall Plant Facility (TPF) in order to research the physiology and genetics of sucrose accumulation under more controlled conditions. The hypothesis proposed was that rapid sucrose accumulation depends on high rates of photosynthesis or preferential allocation of photo-assimilate to sucrose storage or both. To assess the difference in photosynthetic capacity between high and low CCS clones we proposed to use the TPF to measure whole plant photosynthesis of four clones (2 high and 2 low CCS) relative to Q117 for which radiation use efficiency, determined in the field is known.

We suggested that there are two components to the questions about the preferential allocation (partitioning) process. 1) Do high CCS clones regulate expansive growth naturally to divert photosynthate to stored sucrose ? and 2) Do high CCS clones respond more to ripening stimuli like reduced moisture and temperature? If genetics, low moisture and low temperature each use the same source/sink mechanism to enhance sucrose accumulation then the effects of each will not be additive and we should be able to get high CCS from a 'naturally' low CCS clone. If these effects *are* additive then CCS is not entirely controlled by the source/sink process and we will have to elaborate or reject our hypothesis.

We proposed measuring stalk and leaf growth under well watered conditions to determine whether high CCS clones regulate expansive growth without any ripening stimuli or not ('natural' conditions). We will also compare these clones under the ripening stimulus of a low watering regime to determine if the high CCS clones are that way because they respond better to the water stress stimulus than low CCS clones. We will be able to determine the extent to which the CCS trait is 'natural' or dependant on ripening stimuli.

The panel was supportive of all these suggestions probably because many had been discussed with several panel members previously. One member urged us to look at temperature as a ripening stimulus as soon as possible.

3. Notes on workshop on 16 Oct. 07 to assess physiological basis of variety improvement

Results from all experiments conducted in the project to date were presented at the workshop on 16 October and a critical part of the workshop was to assess progress towards a physiological basis of variety improvement. The workshop acknowledged that considerable progress had been made toward understanding, documenting and modeling source – sink control of sucrose accumulation but that there was nothing yet that could be crystallized out for use in breeding or selection. The breeders present agreed that more knowledge about sucrose physiology was useful even if not directly for selection procedures. A firm suggestion was made that rather than looking for traits to select for we should be looking for those to discard which may be easier and more useful in the early stages of selection. The results of CSE014 experiments highlighted the role of stalk population in sucrose accumulation and there was substantial debate over the role of plant anatomy including stalk population, diameter and length in both sucrose and lodging attributes. Breeders would value any indication of

anatomical traits that confer low CCS or lodging tendencies in sugarcane clones so that clones with these traits can be discarded early in the selection process.

Since the rankings of the four clones studied in the TPF were the same in the field and in the glasshouse in regard to both sucrose content and shoot numbers it may be possible to predict performance in the field from glasshouse studies. A firm recommendation from the steering panel and other expert breeders and physiologists was that we need to consider many more clones than the four that have been used thus far. A suggestion was made that we consider high and low CCS categories from extremes of segregating populations as before but increase the numbers in each category to 30 or more. Interest in the individual clones would then be sacrificed for interest in the general nature of high and low CCS categories. This approach aligns with the molecular work where CCS is related to a large number of markers in a large number of clones.

The next two experiments that have been planned and initiated will allow the project team to explore further the source-sink hypothesis for sucrose accumulation in as many as 16 clones but after that we will consider the new direction suggested by the panel to change emphasis from a mechanistic to a statistical approach.



Delegates at the annual CSE014 physiology workshop in Townsville, 16 October 2007

4. Interim assessment of physiological basis of variety improvement – 1 Oct 08.

The model for whole plant sucrose accumulation as presented in the paper submitted to Australian Journal of Agricultural Research (AJAR) and the recent model of sucrose accumulation by internodes of sugarcane developed by Dr Singels are bringing us closer to identifying sub-traits for sucrose accumulation in sugarcane. The paper submitted to AJAR presents evidence to indicate that photosynthesis in high- and low sucrose clones is similar and that there is no limitation in the storage tissue of low sucrose clones for high sucrose content. Differences between clones were found in their responsiveness to water stress which reduced expansive growth more than photosynthesis thus altering the source-sink balance to a lesser extent in low than in high sucrose clones. Differences in high- and low sucrose clones were also found in the morphology of plants such that high sucrose clones produced fewer stalks than low sucrose clones. Low sucrose clones allocated more dry matter to leaves than high sucrose clones possibly to maintain a minimum leaf area for each stalk. It is conceivable that

differences between high- and low- sucrose clones may be explained by source-sink attributes as presented in the AJAR paper and that the biochemistry of tissue which is up- or down regulated for parts of the sucrose accumulation pathway may be an effect rather than a cause of the difference. These are novel observations from a whole plant perspective on sucrose accumulation in sugarcane.

Dr Singels' model identified other sub-traits that distinguish high- from low- sucrose clones albeit supported by only four clones. Leaves of low sucrose types emerged slower than those of high sucrose types but tillers emerged more rapidly in low sucrose types. Biomass accumulation was lower for low sucrose types, particularly for KQ97-2599, than for high sucrose types. The leaf partitioning coefficient was greater for low than for high sucrose types. Interestingly the maximum storage capacity was not distinctly higher for high sucrose types.

When this model is improved and validated using other datasets we will be able to assess ideo-types in silico.

5. Mini-workshop on sucrose accumulation - 7 Oct. 08.

A mini-workshop on sucrose accumulation traits and modelling held on 7 October 2008 at, QPB, St Lucia, Brisbane, 900 to 1230. Present at the meeting were, Drs Graham Bonnett, Scott Chapman, Rosanne Casu, Anne Rae, Meredith McNeil, Karen Aitken, Abraham Singels and Geoff Inman-Bamber. Later that week on 9 October we conducted informal discussions with Prof Graeme Hammer, Dr Scott Chapman, Dr Fernandez Dreccer and Dr Karim Chenu at DPI&F and their informed comments on our work are included.

Experimental results

At the QBP workshop, Geoff Bamber presented recent results on two high and two low CCS clones grown at high and low temperature and under minimal and moderate water stress. Preliminary results of an experiment with 8 high and 8 low CCS clones grown at high and low temperature were also presented. The presentation and intervening discussion brought the group up to date with project findings thus far. There was interest in the observation that the source-sink manipulation by climate (water and temperature) and genetics, involved shoot or tiller numbers. Scott Chapman said that recent findings indicate that stem sugars accumulate more slowly in wheat and sorghum lines with strong rather than weak tillering tendencies in agreement with our model that high population sugarcane clones accumulate sugar slower than low population clones. We discussed source-sink attributes of low and high tillering tendencies as well as competition effects of differing tillering types. In sorghum where tillers are removed, stems of remaining plants can thicken and have very high sucrose contents. In our work, removal of tillers had no influence on sucrose content. This does not necessarily undermine the model (now being published) which includes tiller or shoot number to explain variation in sucrose content in sugarcane clones. This is because genes for tillering may be linked to those for increased allocation of assimilate to leaves rather than stalks as was observed in the low sucrose clones in the Tall Plant Facility (TPF) experiments. It is likely that high population clones are that way because they 'ensure' that enough assimilate is allocated to leaf growth in each of a large number of tillers which would otherwise die if leaf expansion could not be maintained. Artificially removing these tillers does not necessarily alter this type of allometry which seems to be inherited along with tillering propensity.

Karen who works with sugarcane markers and QTLs indicated that markers for tiller number and sucrose content did not often co-locate. Also Karno's PhD work found little association between tiller number and CCS amongst a large number of clones from mapping populations. The analysis was done on relatively small plants which may not have completed the tillering process, nevertheless there is contradictory evidence in sugarcane regarding

tillering and stalk sucrose content. However work in other crops seems to support our observations that tillering is related to sucrose accumulation through source-sink (supply and demand) processes.

We discussed the role of roots which were not considered in the experiments presented by Geoff. It is possible that roots of low CCS clones could demand more assimilate than those of high CCS clones but this may not be observable in the pot experiments. Geoff's results on photosynthesis excluded carbon exchange (CE) in the dark although this was measured. It was suggested that respiration be considered now that the background respiration from the potting mix has been established. Clonal differences in photosynthesis, not apparent from daytime measurements, may be significant when dark CE is considered

Scott suggest that we look at leaf blade to leaf sheath partitioning because they have found that drought resistant wheat cultivars tend to economize on assimilate by producing lighter sheaths and the assimilate thus saved is used to fill the grain. It is possible that high CCS clones do something similar in response to water stress. If this is so drought resistance and CCS could be correlated.

Modelling

Abraham presented progress on modelling sucrose accumulation at the internode level based on the results of Geoff's 4 clone x 2 water regime experiment in the TPF. The group expressed that Abraham had done an excellent job in capturing the dynamics of assimilate partitioning within the stalk and between stalk and leaves. Some additional considerations were offered. Abraham had assumed that assimilate allocation to leaves ceased when leaf elongation ceased but this is not true and leaves will gain in mass slightly after elongation ceases. Abraham used different distribution functions for internode elongation and for fibre deposition and Graham suggested that they should be linked in order to capture the rapid slow-down in cell expansion observed for internode 6. The sucrose content profile was simulated in a realistic manner by the model despite some of these omissions and Abraham was encouraged to test his model or hypothesis using an independent set of data.

Talks with Graeme Hammer were very enlightening and encouraging and it appears that we are not that far behind the genotype to phenotype models that have been developed for maize and sorghum at least in terms of the plant physiology component. There is a remarkable degree of common ground in modelling approaches for sugarcane, wheat, maize and sorghum. All of these approaches deal with tillering and leaf expansion as priority sinks for photo-assimilate and all deal with sucrose accumulation in the stem, some at an internode level, as an overflow of assimilate once priority sinks have been 'filled'. We have adopted a similar approach. The DPI&F group were therefore very interested in our modelling and glasshouse work and we would benefit greatly from closer links to their research efforts.

6. Notes from workshop on 26-27 Nov. 2008

Twenty eight people were invited and 14 attended representing sugar industry breeders, molecular biologists, modellers and physiologists. Geoff Bamber made six presentations about the CSE014 project on sucrose accumulation and there were a number of presentations based on other SRDC funded projects, from BSES staff as well as from Dr Abraham Singels who was visiting from the South African Sugarcane Research Institute (SASRI) at the time. Presentations by Geoff and Abraham (Appendices 2 to 8) were about advances in knowledge of sub traits for sucrose accumulation. Biomass accumulation was remarkably similar in three out of the four clones selected for diversity in sucrose content. Photosynthesis experiments

comparing the four clones indicated that clonal differences in photosynthesis per pot and per unit leaf area of larger plants were small. The data thus led to the conclusion that differences in sucrose accumulation between high- and low sucrose clones was not due to large differences in the rate of photosynthesis.

The clones differed substantially in stalk number and in mass per stalk. The large number of shoots per pot in KQ97-2835 (low sucrose) was associated with high fraction of dry matter in tops, lower stalk dry mass and shorter stalks compared to Q117 and KQ97-5080 (high sucrose). While biomass accumulation in KQ97-2835, Q117 and KQ97-5080 was similar and was slightly lower in KQ97-2599 (low sucrose), photo-assimilate in KQ97-2835 and KQ97-2599 was distributed among about 9-11 stalks per pot for KQ97-2835 and 7-10 stalks per pot for KQ97-2599 compared to only 4-7 stalks for Q117 and KQ97-5080. The most important aspect of stalk numbers was manifest in the large mass of leaf relative to stalk in the high population (low sucrose) clones compared to the low population (high sucrose) clones. These leaves did not result in additional photosynthesis which was evidently rather similar amongst the clones. The presentations suggested that the relatively large amount of new leaf tissue produced by the high population, high sucrose clones placed an additional demand on photo-assimilate in these clones and delayed the accumulation of sucrose in the stalk. The proportion of sucrose in stalk dry matter for low sucrose clones reached levels regarded as close to maximum in previous published research (Berding, 1997; Inman-Bamber et al., 2002). The sucrose content pattern in KQ97-2835 (low sucrose) internodes on 1 May 2007 was similar that of Q117 internodes on 20 Mar. 2007 and it is possible that KQ97-2835 was about eight weeks behind Q117 in allocating photo-assimilate to the stalk. If this was the case then there was no qualitative difference between these clones in regard to sucrose accumulation and the low sucrose content of KQ97-2835 was simply a matter of delay caused by the need to supply more leaf tissue with photosynthate. It is remarkable that two clones selected for low sucrose content should be able to accumulate sucrose equivalent to a highly selected clone (cultivar) like Q117, albeit in only a limited number of basal internodes. As the tested clones were related it suggests that there is little direct genetic control on the maximal amount of sucrose that can accumulate in stalk tissue and that genetic differences, at least in this background, reside more in the morphology of the plant and the way this influences supply and demand for photo-assimilate.

The issue of stalk numbers and sucrose content was debated during workshop. The PhD work of Karno (2007) was noted for the lack of correlation between stalk numbers and sucrose content. Apart from this issue the arguments presented above were not contested by workshop delegates despite the implications for research on sucrose accumulation at the molecular level. If these arguments and the model proves to be valid then the difference between high- and low sucrose clones may well be explained purely on source-sink attributes as represented in this model and the biochemistry of tissue which is up- or down regulated for parts of the sucrose accumulation pathway (Rae et al. 2005; Grof et al., 2007) may be an effect rather than a cause of the difference.

A subsequent experiment with the same four clones, considered the role of stalk numbers in that one treatment consisted of pots containing three plants each with stalk numbers limited to six per pot while another treatment allowed for unlimited tillering and stalk numbers. Artificial stalk number control had remarkably little influence on any of the many attributes measured and there was no indication that tiller removal would increase sucrose content as was suggested by the earlier experiment. We argued that the tiller/shoot number and allocation of

biomass to leaves could be traits that are linked genetically so that artificial removal of tillers had limited impact on distribution of photo-assimilates because high population (low sucrose) clones are 'programmed' to give preference to leaf development in order to keep many tillers alive, even if they are removed. The workshop delegates agreed that this was indeed possible.

We then presented interim results from an experiment with 8 clones representing the upper end of the range in sucrose content from three mapping populations and 8 clones representing the low end of this large range. The most striking difference between these groups of clones was the manner in which biomass was partitioned between leaves and stalks, confirming earlier results with the four clones that differences in sucrose accumulation could be explained quite simply by the carbon mass balance and the sink strength for photo-assimilate being stronger for the development of leaf mass in the low sucrose than the high sucrose clones. Again there was no objection to this possibility from workshop delegates.

We also presented the first set of results for the new project CS023, where single leaf photosynthesis of 10 high sucrose and 10 high fibre clones had been measured repeatedly. Fibre content of the high fibre clones was as high as 30% and CCS for the high sucrose clones (mainly selected varieties) was up to 15% when the plant crop was harvested in June 2008 by Dr Jaya Basnyake for BSS305. Photosynthesis, stomatal conductance and transpiration efficiency all differed significantly between the clones regardless of the fibre or CCS ranking, however high CCS clones were less variable. Prof Bob Lawn (JCU) felt that this result made sense because the high sucrose clones were mainly highly selected commercial varieties while the unselected high fibre clones included some with low photosynthesis rates which would preclude them from selection in the normal plant improvement process.

There was general agreement at the workshop that much had been learnt about sucrose accumulation in the project but that the new knowledge was not ready for implementation in current plant improvement practices.

7. Notes from workshop on 5 Mar. 2009

A workshop was held at SASRI on 5 March 2009 to discuss progress in the understanding of sucrose accumulation from the molecular to the whole plant level. The workshop was intentionally small, attended by seven scientists representing a range of disciplines from molecular biology, plant and crop physiology and modelling. The workshop served to compare the latest progress/new knowledge of source-sink relations, biomass partitioning and sucrose accumulation in sugarcane against a benchmark of a similar workshop held at SASRI in July 2007. The purpose of the workshop was to share the latest thinking on processes and mechanisms involved in genetic and environmental control of structural growth and sucrose accumulation. Only two presentations were arranged one representing work at the whole plant to organ levels and the other representing work at the molecular to plant organ levels. The titles were:

1. Latest concepts of environmental and genetic control of biomass partitioning from modelling research presented by (Singels and Inman-Bamber).
2. Physiological processes that may participate in regulating sucrose accumulation (Watt).

Those present were:

Dr Derek Watt, Head of the variety improvement program at SASRI
Dr Jens Kossmann, director of the Institute for Plant Biotechnology (IPB) in Stellenbosch.
Dr Maurits van Berg, Head of the Resource Optimisation program at SASRI
Dr Abraham Singels, Principle agronomist at SASRI
Dr Michiel Smit, Crop scientist
Mr Ewald Albertse, resident biochemist and PhD candidate
Dr Geoff Inman-Bamber, Principal Research Scientist, CSIRO.

Flows of mass, energy and information about transforming CO₂ to sucrose were outlined by Geoff using the schematic in Fig 1, most of which has been included in the internode model developed by Abraham. Abraham explained the details of partitioning dry matter (DM) amongst internodes and how we selected fixed partitioning functions rather than linking partitioning directly to changing demands by the various sinks for photo-assimilate in the plant. This point was debated, with Maurits suggesting that sink driven partitioning would be a more elegant approach. Geoff agreed with that but countered with the argument that partitioning could be highly conserved ('hard wired') and that empirical data for partitioning between leaves and culms may be very useful in understanding how clones vary in sucrose content (SC). The most striking difference between high and low sucrose clones (apart from their sucrose content) in the CSIRO sucrose accumulation research, is that high sucrose clones allocated more DM to stalk fibre than to leaves. This and a few other traits explain the difference between high and low SC in the model represented in Fig 1. The question is whether we need to delve further into sub-traits for sucrose accumulation or have we already identified mechanisms for genotypic differences in this regard. Nobody was convinced it was that simple. Nevertheless Abraham could and did show how well his model explained differences in sucrose accumulation in whole plants and internodes using simple partitioning coefficients and clonal differences in response to water stress and temperature.

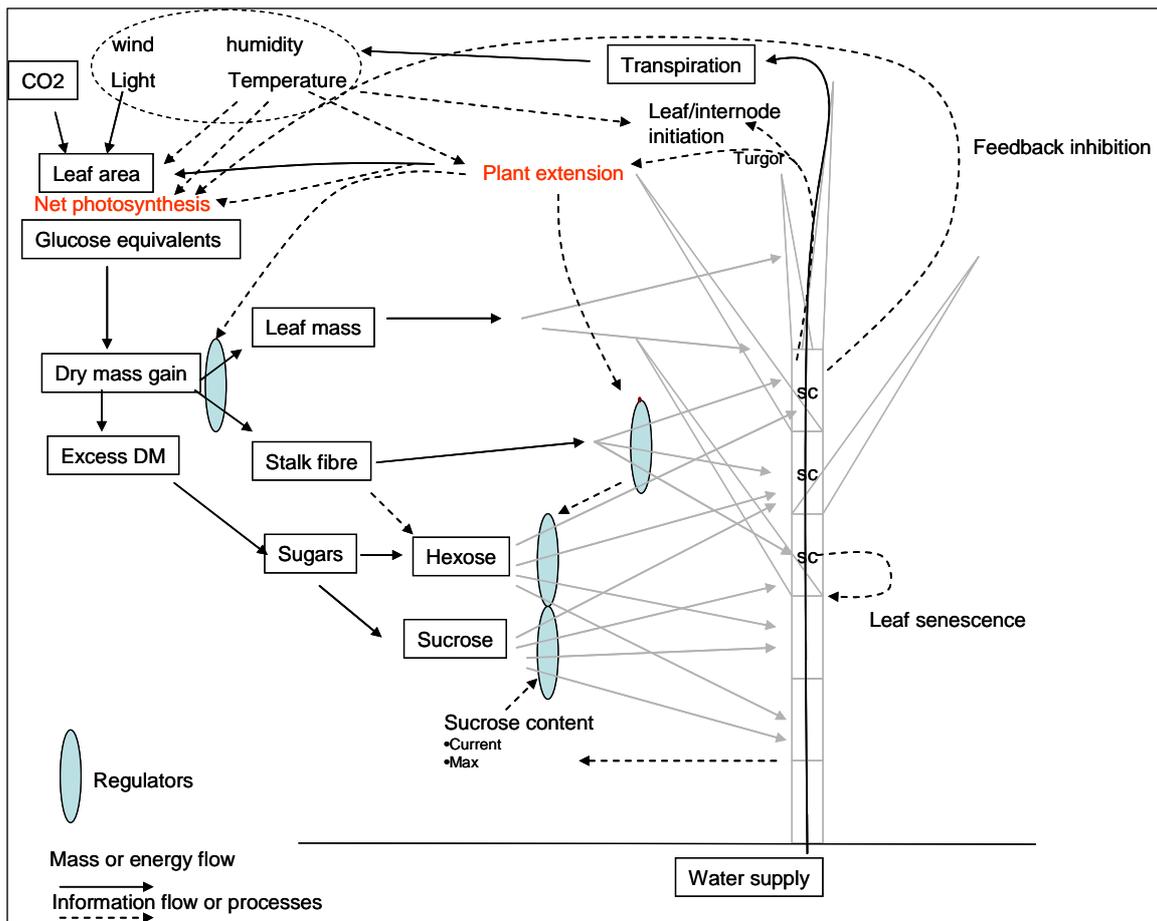


Fig 1. Flows of mass, energy and information for Abrahams model of sucrose accumulation in internodes of sugarcane.

Derek then briefly traced the history of the search for biochemical mechanisms and genes associated with sucrose accumulation. Three plant level components in the sucrose storage process, leaves (photosynthesis), phloem transport and storage in the stalk have all received some attention by the biotech group at SASRI but processes within the stalk have received by far the most attention. Initial attempts to engineer increased sucrose accumulation included down-regulation of single enzymes such neutral invertase involved 'futile cycling', PFP, adolase, enolase and Fru 2,6-bisphosphatase involved in glycolytic flux. Derek outlined new generation strategies now undertaken largely by IPB on a contract basis. These included regulation of enzymes in tandem (stacked transgenes) such as PFP and UDP-Glc DHase and by increasing vacuolar sequestration involving translocating pyrophosphatase and sucrose transporter genes.

It is fair to say that interventions by molecular means of components in the stalk (membrane transport, sucrose turnover, glycolytic flux, fibre biosynthesis and biophysical constraints) have not revealed the causal processes underlying differences between high and low SC clones. The wealth of knowledge gained in recent years of these processes has not made it easier to modify sugarcane biochemistry to encourage more sucrose to accumulate. The focus of Derek's group has turned to feedback inhibition of photosynthesis by sucrose with a number of publications in high impact journals demonstrating that this could be a rate limiting step worth modifying by gene transfer techniques. Derek's work with Alistair McCormick (now at Oxford) showed that photosynthesis slowed when sucrose accumulated in the leaf above a cold girdle which restricted translocation via the phloem. Leaf photosynthesis increased when other

leaves were removed or shaded indicating that photosynthesis is normally restricted by an over supply of assimilate, possibly related to high sucrose levels in the stalk. Further work will concentrate on the signalling processes which could then be down-regulated thus allowing high rates of photosynthesis to continue and higher levels of sucrose to accumulate.

Derek was interrupted many times for questions and discussion. Sucrose concentration in his work is expressed in terms of moles of protein whereas the model presented by Abraham envisaged storage in terms of dry stalk mass (DM) or fibre mass. The use of different units makes it hard for integration of the molecular and whole plant approaches. Evald explained that moles of protein were used to reflect the number cells in internodal tissue and so base sucrose concentration at more fundamental level in terms of storage efficiency than the DM approach. Clearly we need to be able to convert between the two types of units and two methods for doing this were discussed: 1) the dry matter content (DMC) of internodes sampled for HPLC analyses can be estimated reliably from DMC of internodes on either side 2) additional tissue for DMC determination could be taken from internodes that are sampled for HPLC analyses as we do at CSIRO.

Hexose accumulation and distribution in Abraham's model depends of the rate of fibre deposition and reference was made to the PhD work of Jan Bekker who is looking at the biosynthesis of fibre from hexose.

Derek explained the yeast source-sink (supply:demand) model by Hofmeyr (1998) and Hofmeyr & Cornish-Bowden, (2000) to show how sink demand could often control the rate of photosynthesis in order to regulate an intermediate metabolite such as sucrose in the phloem. Abraham was well aware of this work and felt that Hofmeyer's mathematical model could be helpful in the overall model of sucrose accumulation in internodes.

We also discussed the processes that may be involved in sucrose signalling and reference was made to Trehalose – 6 – phosphate as (T6P) a metabolite that is involved in source- regulation at hormone like concentrations in many crop species. We were referred to the work of Marne van der Merwe who is studying this topic for her PhD.

Michiel illustrated how source-sink regulation works in soybeans in regard to the production of flowers, pods and seeds requiring increasing amounts of photo-assimilate. Photosynthesis was often the rate limiting step in his example.

Future work and opportunities for collaboration

The final part of the workshop was to consider current and future projects by the four groups represented (SASRI Agronomy, SASRI plant improvement, CSIRO and IPB).

Opportunities for collaboration:

- 1) IPB have developed a modified version of NCo310 which is down regulated for glycolysis and PFP activity. These plants will go to field trails in April and this provides an ideal opportunity to test Abraham's model on highly modified plants. Biomass sampling and partitioning in these plants was suggested. We would have come a long way in the unravelling of the manipulable pathways for sucrose accumulation if the modified plants can be described at the whole plant and molecular levels simultaneously.
- 2) Geoff's new project on feed back inhibition of photosynthesis of leaf segments and whole plants, complements several SASRI projects on the subject. Geoff will send the

project outline to Evald (Derek has already discussed the project with Geoff) and Evald will suggest ways in which this project could link with his PhD work.

- 3) Riekert van Heerden (who could not attend) is an expert in chlorophyll fluorescence. Riekert will continue discussions with Geoff to see how his expertise could contribute to the new project with Geoff considering a possible visit for Riekert to Australia to help with this project (depending on success of funding applications of course).

8. Contribution of project to breeding technology evaluated against baseline

In milestone 3, principles and practices of the current crossing and selection strategies were documented using a questionnaire completed by 11 members of the BSES-CSIRO-CSR Sugarcane Variety Improvement Program. While many people in this program notably Drs Phil Jackson, Nils Berding, Prakash Lakshmanan and Graham Bonnett have been intimately involved in the project we cannot claim that anything has changed in the breeding program as a result of this work. The project offered a fresh challenge to the molecular biologists to look at the whole plant rather than just the tissue in which sucrose accumulates. The project has taken the argument for a top down and bottom up approach, to those involved in breeding and selection, molecular biology and plant physiology to think about plant improvement and production as a whole system. Thus those working on feedback inhibition by sucrose were encouraged at the SASRI workshop to think of other reasons why sucrose accumulation declined after removing leaves, before going to the expense of identifying processes and genes involved in feedback inhibition. Those identifying genes involved in up or down regulation of sucrose accumulation were also challenged to look at the whole plant to ensure that they are working with the causes of sucrose accumulation rather than the effects of some other cause as suggested by Inman-Bamber et al., (2009). The internode sucrose accumulation model built by Dr Singels was offered as a framework on which to build our knowledge of the sucrose accumulation process. The model explains sucrose accumulation by maintaining the mass balance of carbohydrate generation (photosynthesis) and distribution to the various plant components and to each internode. We believe that this framework provides the best chance for a comprehensive understanding of the complex interactions between the climatic and cell environments in the production of sucrose. The impact on the breeding program will then occur through the capability to evaluate sub-traits of sucrose accumulation in the context of climate variability over space and time, in the Australian sugar industry.

Appendix 15

First Brazilian Symposium on Sugarcane Ripening Botucatu, May 2008 Whole plant and crop perspective of the physiology of sucrose accumulation in sugarcane

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Introduction

In the sugarcane industry high sucrose content in cane stalks is a high priority for farmers, and consequently for breeders and agronomists as well. In the Australian sugar industry as in many others, the value of the crop delivered to the mill is measured in terms of commercial cane sugar (CCS) in which sucrose content is the dominant factor. An increase in sucrose yield due to improved CCS is up to 1.8 times more valuable than a sucrose yield increase due to improved cane yield (Jackson *et al.* 2000). This is because increased cane yield attracts marginal costs in harvesting, transport and milling whereas an increased CCS does not. Unfortunately in Australia, recent improvements in sugar yield have been achieved through cultivars with improved cane yield rather than improved CCS (Jackson 2005) and this is a common feature in sugarcane industries around the world (Moore 2005, Inman-Bamber *et al.*, 2008).

A large effort is now underway to find alternative methods to develop cultivars with improved sugar content. One method is to increase sucrose content by incorporating more accessions of the progenitor basic germplasm, facilitated by molecular markers (Aitken *et al.* 2006). Another is utilising cell biology and genomic approaches to identify genes that are involved in, or correlated with sucrose accumulation (Casu *et al.* 2004, 2005; Rae *et al.* 2005; Watt *et al.* 2005).

This paper provides a brief overview of the research aimed at understanding sucrose accumulation at the crop and whole plant levels rather than at the cellular or molecular levels. It is suggested that such an understanding will expedite the development of high sucrose varieties through conventional or molecular approaches to plant improvement, will assist growers to manage their crops for enhanced sucrose content and will assist with testing of chemical ripeners.

Crop phenology (developmental stages)

Sugars accumulate in the stalk as soon as internodes start to elongate and there is a well established pattern of increasing sucrose content (SC) from the youngest to the oldest internode (van Dillewijn, 1952; Fernandes and Benda, 1985; Robertson *et al.*, 1996). Our own was grouped by stalk mass rather than age (Inman-Bamber *et al.*, 2002) (Fig 1). At the base of the stalk, SC on a dry matter basis (SCd) increased markedly with the first two 50-g increments

in stalk dry mass and reached a maximum of about 0.55 g/g in heavier stalks. The decline in SC toward the top of the stalk was similar for each stalk mass class. It is therefore the length of stalk with near maximum SC that determines the SC of whole stalks.

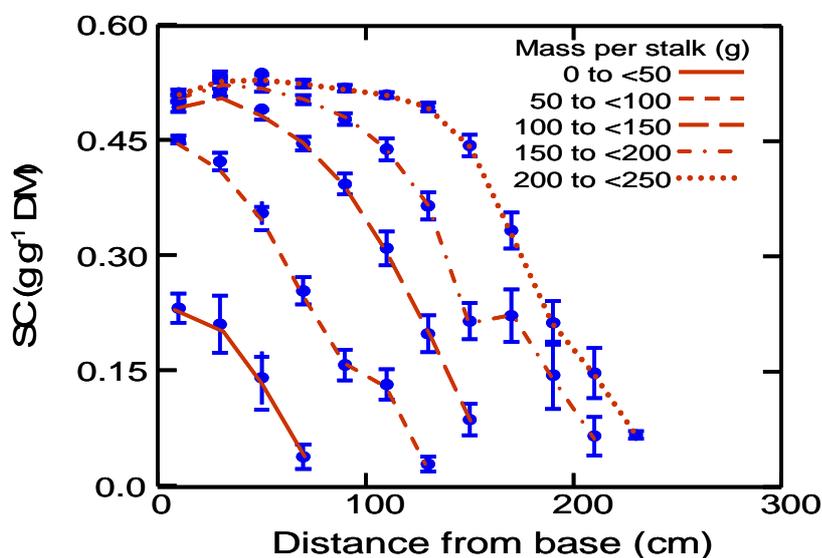


Fig 1. Mean sucrose content on a dry mass basis (SCd) of 20-cm stalk segments from cultivar NCo376, grouped by stalk dry mass, versus stalk height. Bars are 2X standard error of the mean (Inman-Bamber et al., 2002).

Stalk height and crop age are obviously highly correlated with cane mass and these could replace cane mass as a grouping variable in Fig. 1 with similar effect. Maturation in sugarcane could be described in two phases, one in which SC of basal internodes is increasing and the other in which SC of basal internodes has reached a maximum. In the second phase, further increments in SC of whole stalks depends mostly on ripening of distal internodes. Once the crop is through the first phase, seasonal variation in SC of whole stalks is largely due to partitioning to sucrose in distal internodes mediated by factors such as water and nutrient stress and temperature which affect expansive growth more than photosynthesis.

Seasonal effects

Data in Fig. 1 suggests that there is little change in SC of basal internodes, once a ceiling value of about 0.55 is obtained for stalks with a dry weight of more than 150 g. However, seasonal effects on SC in 0 to 20 cm and 20 to 40 cm segments *were* evident in stalks weighing more than 150 g when age effects were shown to be minimal. SC of basal sections of the stalk was lowest in Autumn, highest in Spring and it decreased during Summer (Fig. 2). The decrease in mean SC of the two basal segments of large stalks during summer (Fig. 2) could mean that stored sucrose was remobilised to support renewed growth in summer, amounting to a real loss in accumulated sucrose mass. A reduction in SC in the base of stalks could arise from net export of sucrose from storage tissue, from sucrose hydrolysis or from deposition of other cell components such as suberin or lignin. Products of sucrose hydrolysis would result in decreased purity unless these too were exported to growing regions of the plant. Net export of

sucrose and hexoses is thus evident from Fig. 2 where purity (sucrose/brix) remained at high levels as SC decreased. The mass of sucrose in basal segments was subject to the same seasonal variation as SC (Fig. 2) indicating that changes in SC were due to changes in sucrose mass per segment rather than dilution by other solutes and cell wall constituents. A similar reduction in SC in basal internodes was observed when 12 and 24 month old primary stalks were sampled at two sites in New South Wales, Australia (Hughes and Muchow, 2000). Export of sucrose from basal segments would then augment the 'dilution' concept described above.

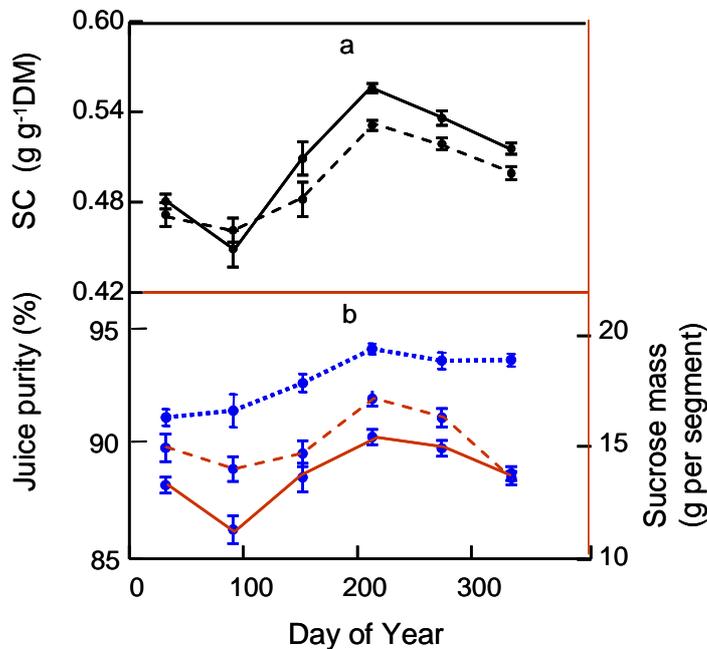


Fig 2. a) Sucrose content of dry matter (SC) and (b) sucrose mass of stalk segments, 0 to 20 cm (dashed line) and 20 to 40 cm (solid line) and juice purity of 0 to 40 cm segment (dotted line) of NCo376 for stalks weighing more than 150 g dry mass and sampled at 2-month intervals. Stalks were segmented from the base upwards. Bars are 2X standard error of the mean (Inman-Bamber et al., 2002).

The seasonal effect on SC over and above the developmental effect can be seen in the slope of the sucrose mass vs stalk mass relationship which is a measure of the allocation to sucrose from photo-assimilate during the season (Fig. 3). These data are from South Africa (Inman-Bamber et al, 2002) where SC reaches a peak usually around August - September when conditions are cool and dry and stalk elongation is slow. Under these conditions nearly 60% of stalk dry matter was allocated to sucrose in the variety NCo376. Minimum amounts of stalk dry matter were allocated to sucrose accumulation when stalk elongation rates were presumed to be rapid from December to March which is normally the case.

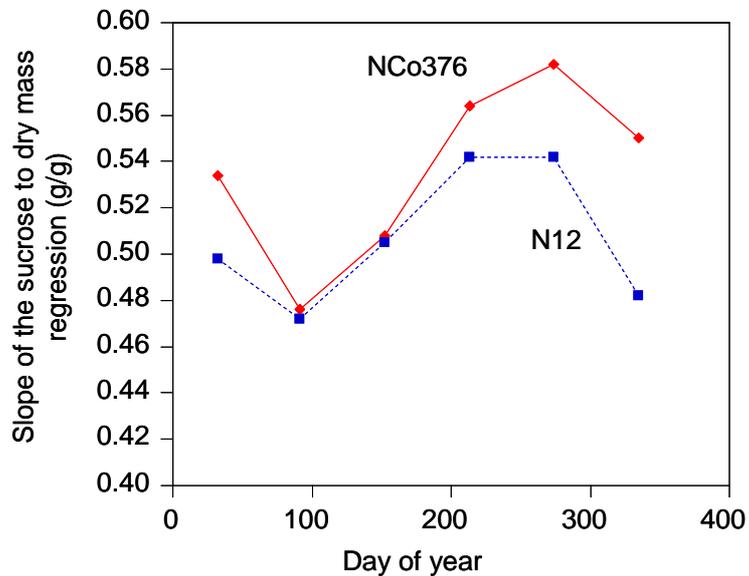


Fig 3. Slope of the sucrose mass vs stalk dry mass regression for stalks sampled at different times of the calendar year for two varieties grown under rainfed conditions in South Africa (after Inman-Bamber et al., 2002)

McDonald (2006) was able to separate developmental and seasonal effects on SC by ratooning and sampling crops at 8 week intervals throughout the year in an irrigated experiment carried out in the Burdekin in Queensland Australia. The seasonal effect dominated when crop age was 40 to 52 weeks, and the age effect was prominent in younger and older crops (Fig. 4). In January when moisture and temperature conditions are favourable for rapid stalk growth, sucrose content of fresh stalk mass (SCf) was low in crops 52 weeks and younger but was considerably greater in the 56 and 64 week old crops (Fig. 4). It is possible that stalk elongation (not measured) was reduced in the older crops or that stalks were long and heavy enough for the low SC of recently developed internodes to have minimal impact on the SCf of the whole stalk. The reverse would have been true for the 32 week old crop in December (Fig. 4).

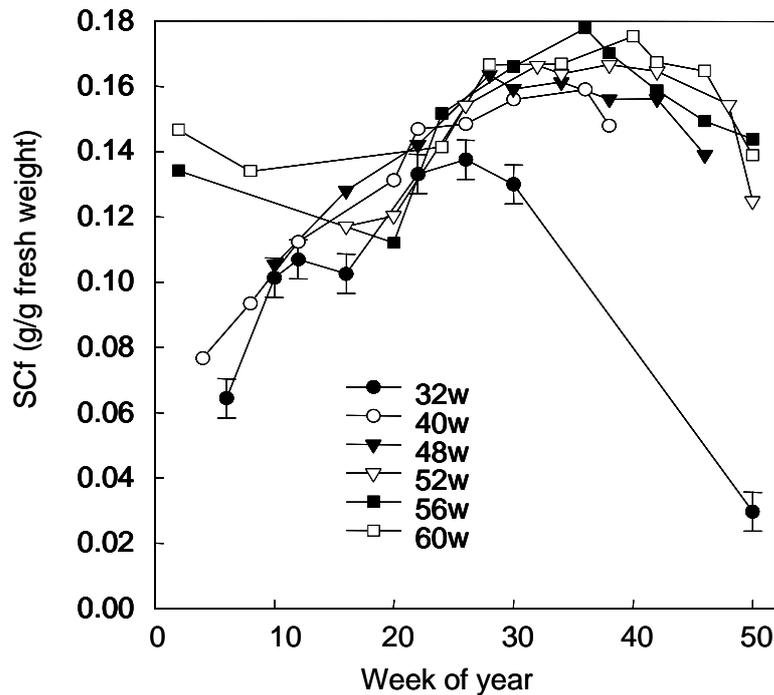


Fig 4. Sucrose content of fresh stalk mass of Q96 sampled in ratoons starting at different times in the year and sampled at a range of ages (week) throughout the year (after McDonald, 2006).

Effect of water regime

The association between water regime as governed largely by rainfall, irrigation and evaporation, is of particular interest in counties where irrigation of sugarcane is widespread and where rainfall variability is high. Many experiments with varying water regimes have led to the conclusion that sucrose accumulation is highly dependant on relative rates of photosynthesis and expansive growth. In one experiment where irrigation was suspended for about five months before harvesting in October, differences in SC between treatments were evident over the entire length of the stalk (Fig. 5a, Inman-Bamber, 2004). At the end of the experiment the difference between treatments was almost entirely in the top 500 mm of stalk. It is interesting that a long period without irrigation did not necessarily lead to the highest dry matter content. Thus the control of water content of stalks appears to be more a case of active replacement of water with dry matter constituents, rather than passive desiccation of stalks.

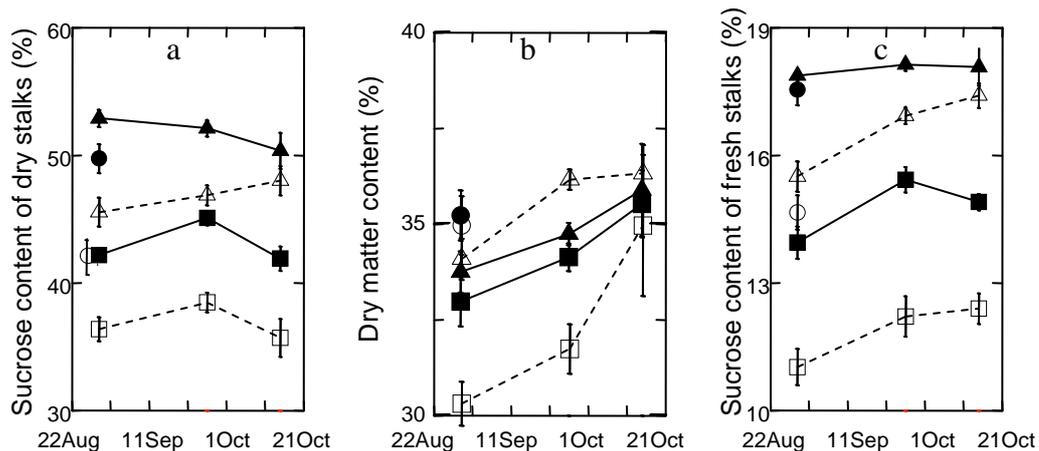


Fig. 5. a) Sucrose content of stalk segments on a dry mass, b) dry matter content and c) sucrose content on a fresh mass basis. Top 500 mm of stalk (□,■), bottom 500 mm(○,●), remainder (△,▲). Solid lines and open symbols are for the *wet* treatment, closed symbols and broken lines are for the *dry* treatment. Bars denote 2x standard error of the mean (Inman-Bamber, 2004).

In another experiment potted plants were irrigated fully (*wet*) or partially (*dry*) to limit plant extension rate (PER) without limiting photosynthesis to the same extent (Inman-Bamber, et al., 2008). *SCd* increased with internode age up to internode 10 and tended to remain at elevated levels for older internodes (Fig. 6). Treatment differences were small for internode 2 and greatest for internode 6 in Q138 and internode 8 in Q183 giving rise to a steeper downward gradient in *SCd* toward the top of the stalk (Fig 6). Singels and Bezuidenhout (2002) proposed that ripening responses would be identified in terms of this slope rather than increased in *SC* in the base of the stalk. These data only partly support this hypothesis and it is clear that ripening from partial water stress occurred in all but the youngest internodes.

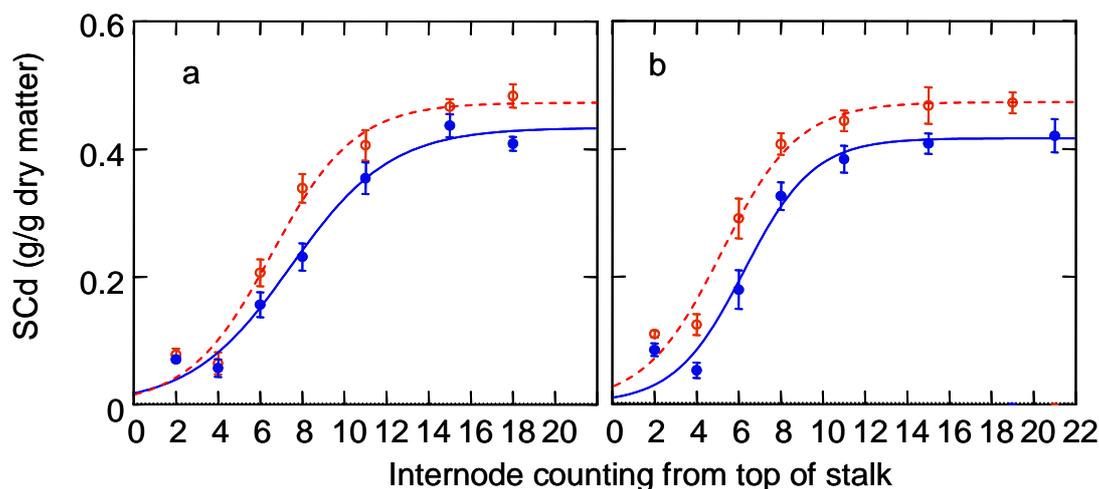


Fig. 6. Mean sucrose content (*SCd*) of dry matter of internodes for *wet* (●) and *dry* plants (○) of Q138 (a) and Q183 (b) after Inman-Bamber et al. (2008)

Botha and Black (2000) reported rates of sucrose accumulation of field grown sugarcane that were greatest in internodes 9 to 11 despite SCd approaching a maximum of about 0.54 g/g in internode 11. If sucrose generally accumulates most rapidly in internodes 9 to 11 then changes in assimilate partitioning are likely to be noticed more in these than in younger or older internodes whether or not these internodes were expanding at the time when source or sinks were altered (Inman-Bamber et al., 2008).

Sucrose content of whole stalks can be increased considerably by moderate water stress. In a rainout shelter experiment, sucrose yield was 11.8 t/ha in the well-watered treatment and 10.7 t/ha in a crop which was denied water for five months (Inman-Bamber and de Jager, 1988). Cane yield was reduced from 108 to 75 t/ha by the latter treatment, but SCf was increased from 10.9 to 14.3 % which nearly offset the large loss in cane yield. In another experiment, normal estate irrigation management was applied to 'wet' plots (Fig 7a) while 'dry' plots received one irrigation before the summer monsoon season but no irrigation at all after that (Fig. 7). SCf was similar for both treatments until mid August when SCf increased rapidly to 17% in the *dry* treatment compared to 14% for the *wet* treatment (Fig 7b). Sucrose yield was significantly greater in the *dry* than the *wet* treatment at this time but differences narrowed thereafter and both treatments produced about 19 t/ha sucrose at the end of the experiment (Fig 7b).

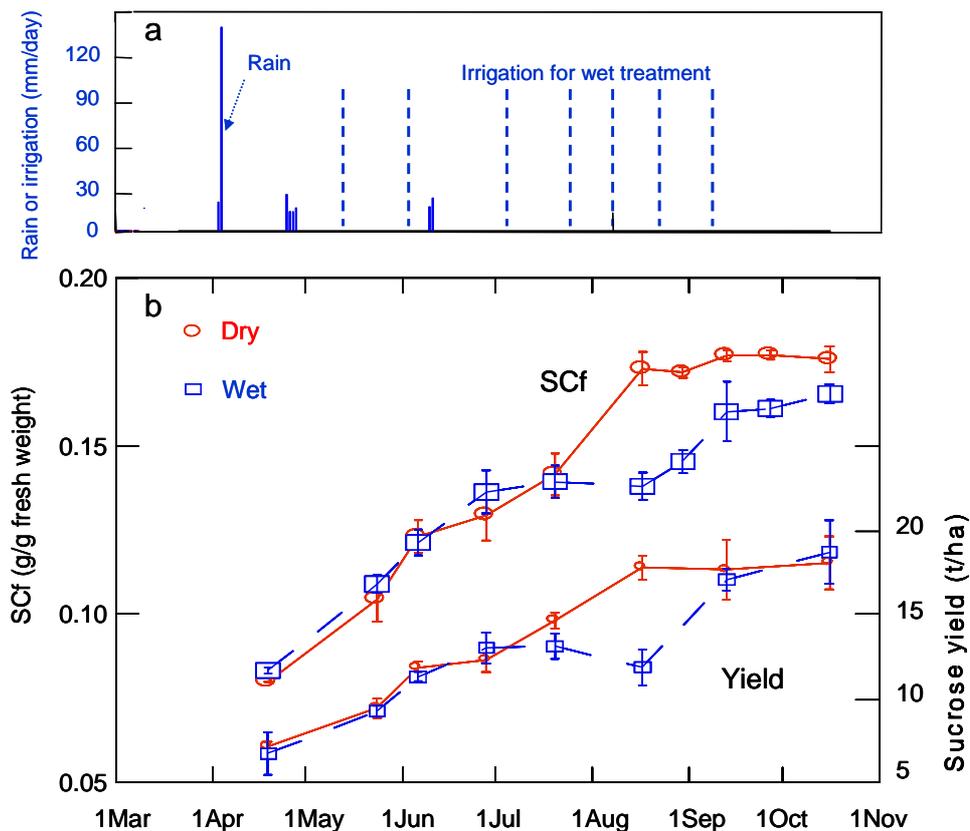


Fig 7. a) Rainfall and irrigation applied to *wet* plots and b) sucrose content of fresh mass and sucrose yield in a November ratoon which was either well irrigated (— —, *wet*) or not irrigated (— —, *dry*) after the *wet* season after Inman-Bamber (2004). Bars indicate 2x standard error of the mean

Source – sink control of sucrose accumulation.

Auxanometers to measure plant extension rate (PER) were fitted in the experiment just described (Inman-Bamber, 2004). Both *wet* and *dry* treatments displayed high rates of elongation during the warmer months of the wet season (Fig 7 and 8) but PER of the *wet* treatment was reduced as minimum temperatures declined during winter. Leaf and stalk elongation of the *dry* treatment practically stopped after mid August (day of year= 230) which is when the SCf of the *dry* treatment was about 4% units greater than SCf of the *wet* treatment (Fig 7).

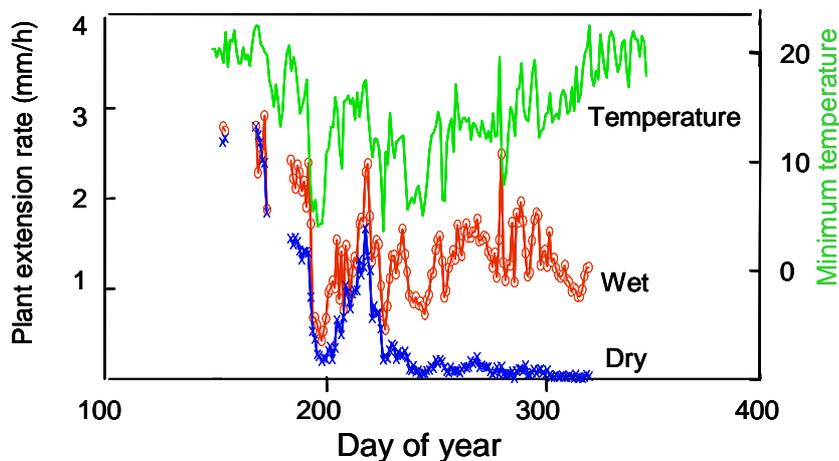


Fig 8. Plant extension rate and minimum daily temperature in an experiment in which some plots were irrigated regularly (*wet*) and others not at all after the wet season (after Inman-Bamber et al., 2002).

In the rainout shelter experiment mentioned earlier where some plots were irrigated frequently and others were denied rain or irrigation for five months, stomatal conductance responded to treatment about two weeks after responses in PER were noted and conductance was reduced only 50% when PER reached zero (Fig. 9). Two months after expansive growth had ceased (PER=0), conductance was still about 1.5 m/s compared with 3 m/s for irrigated plants (Inman-Bamber, 1995, Inman-Bamber and Smith, 2005). SCf was increased by 3.4% units by this treatment. Deep soil and cool conditions were responsible for the slow imposition of stress.

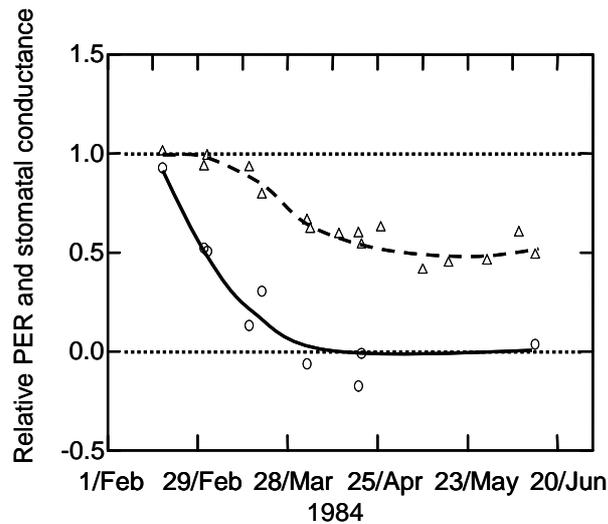


Fig. 9. Plant extension rate (○) and stomatal conductance (△) of sugarcane without rain or irrigation after 31 January 1984, *relative* to PER and conductance of well irrigated sugarcane (from Inman-Bamber and Smith, (2005) reworked from Inman-Bamber, 1995). Lines were fitted by eye.

From Figs 8 and 9 it appears that sucrose content and sucrose mass increases rapidly after leaf and stalk elongation cease provided photosynthesis still continues. Robertson and Donaldson (1998) showed that sucrose content on a fresh mass basis remained higher in dry-down treatments than fully irrigated treatments, even when the yield in the dry-down treatments had been reduced by as much as 50 % by water stress. It is conceivable that, depending on the extent to which photosynthesis is affected, mild water stress could actually increase sucrose yield because assimilate that would have been used for cell wall synthesis (fibre) can be stored in existing stalk tissue. Inman-Bamber et al., (2008) designed equipment and an experiment to determine the extent to which sucrose would accumulate in cane stalks when expansive growth was reduced more than photosynthesis by water stress.

Pot experiment on source- sink control of sucrose accumulation (Inman-Bamber et al., 2008)

The research was conducted on a 'low' (Q138) and a 'high' (Q183) SC cultivar in two temperature controlled and airtight glasshouses (chambers) at CSIRO's Davies Laboratory in Townsville. Potted plants of each cultivar were placed in two chambers of the 'Tall Plant Facility' (TPF) and in one chamber they were irrigated to minimise water stress ('wet' treatment) while plants in the other chamber ('dry' treatment) were irrigated to reduce PER considerably more than photosynthesis (*P*) which was measured for all plants in the chambers throughout each day. Fig. 10 shows PER and *P* during one of these days and in this case PER was zero for four hours in the middle of the day when *P* of wet plants was highest. *P* of dry plants was about half that of wet plants in the middle of the day. This is consistent with results of the rainout shelter experiment where stomatal conductance was reduced only 50% when PER reached zero (Inman-Bamber, 1995).

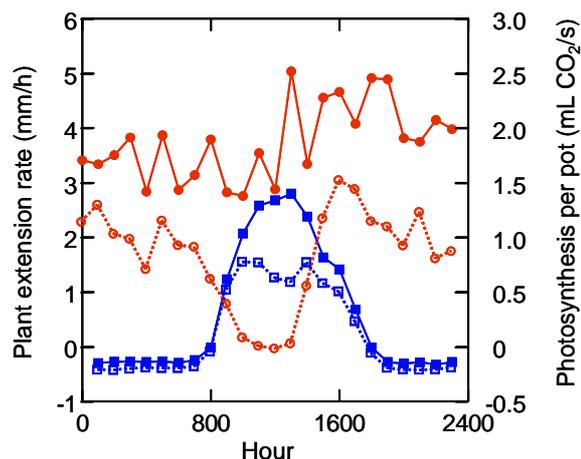


Fig. 10. Plant extension rate (●,○) and photosynthesis (■,□) during 18 April 2006 in potted plants in a glasshouse with abundant (●■) and limited irrigation (○□).

P of young leaves was measured in addition to P of whole plants on some days. Daily mean leaf photosynthesis on 16 March for *wet* plants ($30.2 \mu\text{mol m}^{-2} \text{s}^{-1}$) was significantly greater ($p < 0.001$) than for *dry* plants ($25.0 \mu\text{mol m}^{-2} \text{s}^{-1}$). The difference in mean photosynthesis was smaller on 17 March (33.6 for *wet* vs $31.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ for *dry* plants) but was still significant ($p = 0.002$). Mean PER of *wet* plants during photosynthesis measurements on 16 March was 4.9 vs 0.5 mm/h for *dry* plants and on 17 March the comparison was 4.3 vs 2.4 mm/h . Thus PER and photosynthesis were reduced by 90 and 17% respectively on 16 March, and by 44 and 6% respectively on 17 March, through water stress. Responses in PER and leaf photosynthesis to water stress on 17 March were similar to the mean effect of water stress on PER and whole plant P over the entire experiment.

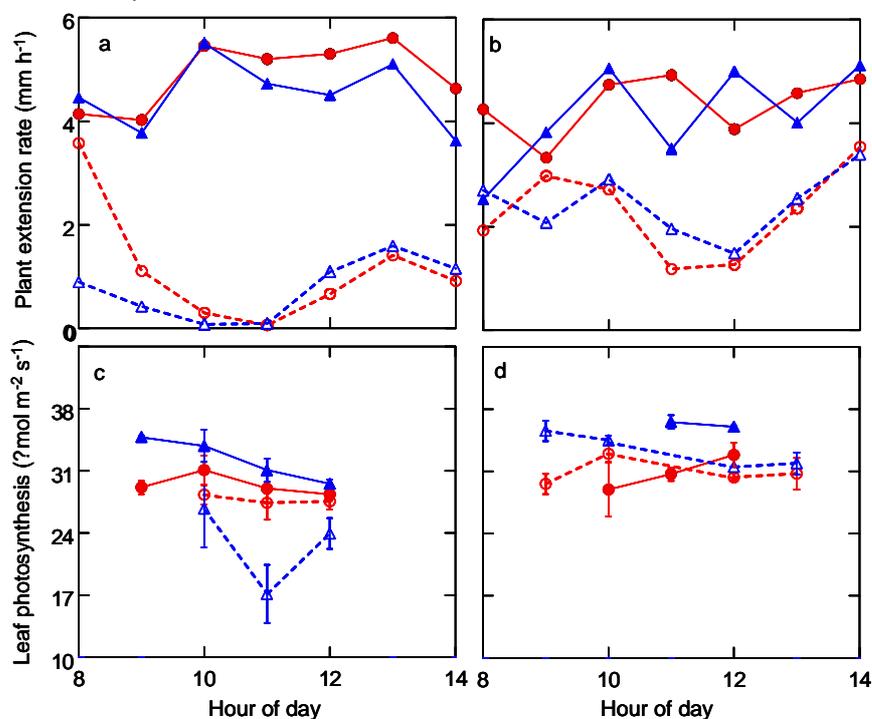


Fig. 11. Hourly plant extension rate (a,b) and leaf photosynthesis (c,d) for well watered (*wet* ●,▲) and stressed (*dry* ○,△) plants of Q138 (●,○) and for Q183 (▲,△) on 16 March (a,c) and 17 March (b,d), after Inman-Bamber et al. (2008).

While the aim was to maintain daily mean PER of *dry* plants 25 to 35 % lower than that of *wet* plants, PER responded so rapidly to changes in soil water content that this aim was only achieved after some trial and error with irrigation scheduling (Fig 12). It is noteworthy that PER of *wet* plants declined from mid March to the end of April even though the temperature regime was the same each day (Fig. 12).

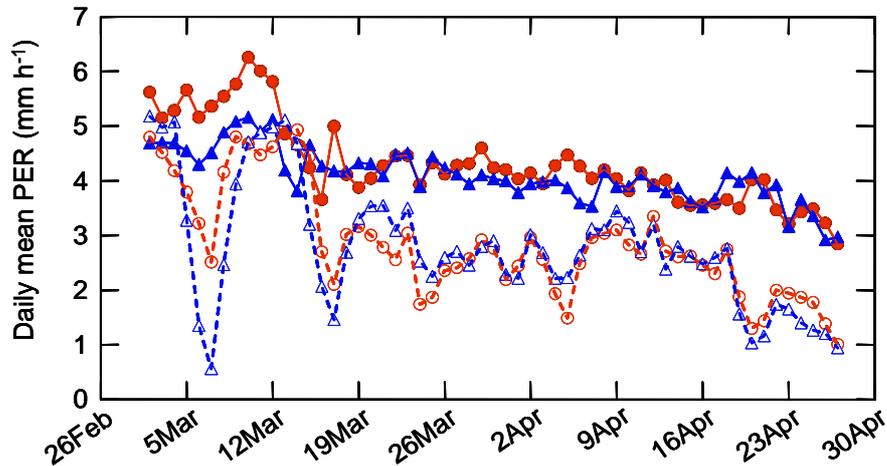


Fig. 12 Daily mean plant extension rate (PER) for well watered (*wet* ●, ▲) and stressed (*dry* ○, △) plants and for Q138 (●, ○) and for Q183 (▲, △), after Inman-Bamber et al., (2008).

By comparing photosynthesis of *wet* and *dry* plants on a per pot basis we have a measure ($P_{rel} = P_{dry}/P_{wet}$) of the effect of water stress on relative photosynthetic or 'source' activity in the *dry* treatment (Fig 13). Similarly by comparing *PER* of *dry* plants with that of *wet* plants ($PER_{rel} = PER_{dry}/PER_{wet}$) we have a measure of the effect of water stress on relative sink activity of the growing stalk and expanding leaves of the *dry* treatment. Apart from one outlier, P_{rel} started at 0.8 and then increased steadily to about 1.0 while PER_{rel} was mostly in the 0.5 to 0.7 range. Mean P_{rel} was 0.82 and mean PER_{rel} was 0.59 so the overall effect of water stress was to reduce photosynthesis by 18% while expansive growth was reduced by 41%. The effect of water stress on source activity was through reduced leaf area as well as reduced photosynthesis per unit leaf area.

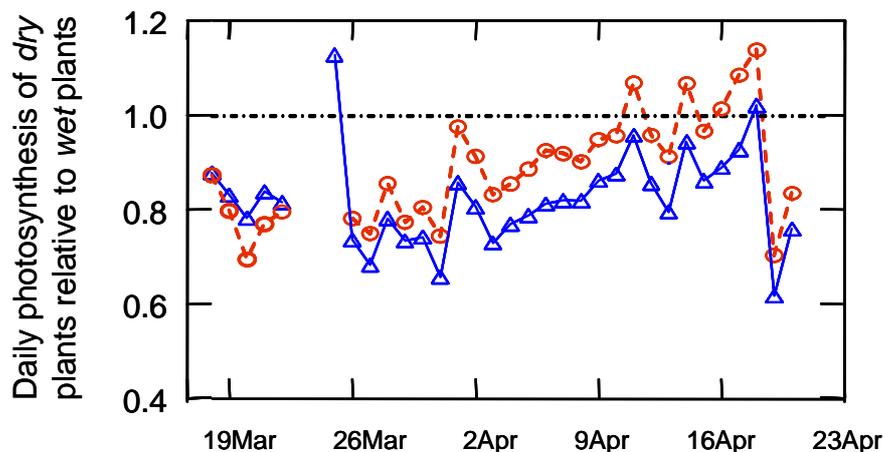


Fig. 13. Whole plant photosynthesis per pot (solid line, Δ) and per m² leaf area (broken line, ○) of 'dry' plants relative to photosynthesis to 'wet' plants. Data were excluded when solar radiation < 10 W m² after Inman-Bamber et al., (2008).

SCd of *wet* plants remained low throughout the experiment because of the rapid rate of leaf and stalk extension which was encouraged by maximum temperatures set at 30 °C (Mean 25 °C) in the TPF. Reduced PER in the *dry* treatment resulted in a 28% increase (24 to 32 %) in SCd after only 4 weeks of treatment. However this difference was not increased as may have been expected over the next 3 weeks of treatment application (Fig 14). This is possibly due to the reduction in PER of *wet* plants despite the continued high temperatures in the TPF. It is possible that ripening in sugarcane occurs not only from the increased length of stalk which contains maximum sucrose content, but because stalk growth rate slows down with age while photosynthesis remains high thus allowing more photo-assimilated to accumulate as sucrose in the stalk. The increase in SCd resulted in a significant increase in sucrose mass (Fig 14).

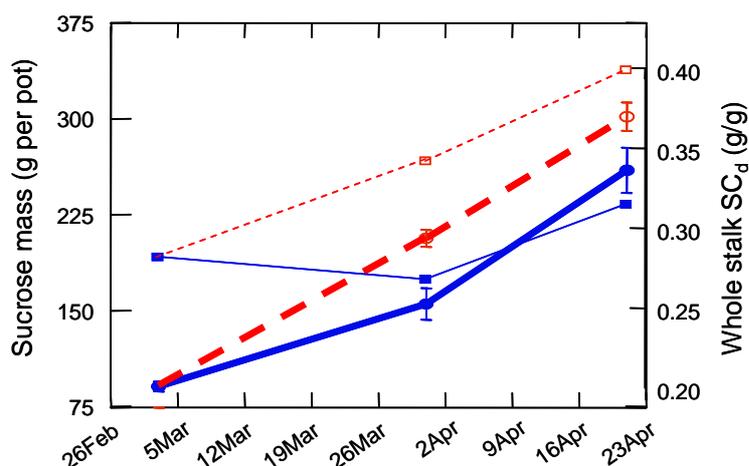


Fig 14. Sucrose content of *dry* mass (thin lines) and sucrose mass per pot (bold lines) for *wet* (•) and *dry* (---) treatments after Inman-Bamber et al., (2008).

Summary of pot experiment results

Water stress reduced total biomass gain by 142 g per pot (19%) and it reduced biomass gain in leaves plus cabbage (tops) by 84 g (37%), in stalks by 58 g (14%) and it increased sucrose mass gain by 41 g (27%). Water stress reduced whole plant photosynthesis by 18% thus largely accounting for the 19% reduction in biomass accumulation and it reduced PER by 41 % thus largely accounting for the 37% reduction in mass of tops. Fibre can be regarded as the main stalk constituent other than sucrose and 109 g per pot (31%) less fibre accumulated under water stress. From this we hypothesise that reduced PER resulted in reduced demand for photo-assimilate by fibre and tops thus allowing excess assimilate to accumulate in the form of sucrose (Inman-Bamber et al., 2008).

Chemical ripeners

Dry matter partitioning is altered by ripeners in a manner similar to water stress. For example the herbicide Fusilade Super (Fluazifop-butyl) inhibits de novo synthesis of fatty acids and in doing so disrupts formation of cell structures requiring fatty acids or lipids (Gronwald, 1991). Donaldson and van Staden (1995) analysed the response of sugarcane to Fusilade in well-watered sugarcane. The production of new leaves was reduced by Fusilade but the total area per stalk was unaffected because senescence of older leaves was delayed by the ripener in these conditions. The water stress treatment reduced the production of new leaves as well as the total green leaf area per stalk. Dry mass of stalk segments was not affected either by ripener or water stress treatments, but sucrose content in the top 200 mm of stalk was increased by as much as 60% by Fusilade, with or without water stress. Water stress and ripener produced remarkably similar increases in sucrose content at the expense of both fibre and reducing sugars. Effects of water stress and ripener were therefore not additive. Growers can use one or the other treatment to enhance sucrose content but not both as they exploit the same source-sink mechanism in repartitioning photo-assimilate.

Summary

Sucrose content increases with crop age largely because the immature internodes at the top of the stalk make a diminishing contribution to sucrose content of the whole stalk as the stalk develops more mature internodes in which the SC is high or near maximum. Seasonal variation in SC is due to variation in the ratio of mature to immature stalk tissue as well as variation in SC of mature internodes.

Water stress can result in an increase in SC even when it reduces yields by as much as 50%. Moderate water stress imposed over several weeks or months can increase SC_f by as much as 4% units and in doing so can off-set large reductions in cane yield.

The manipulation of dry matter partitioning to new stalk and leaf growth through irrigation, temperature and possibly N or chemical ripeners can lead to substantial increases in sucrose content and possibly sucrose yield as well.

A good understanding of the source-sink mechanisms giving rise to this result could lead both to increased sucrose yields and water savings. Conversely misunderstanding of the ripening response could lead to losses in sucrose yield. Thus sucrose yield losses can arise by paying too much attention to sucrose content and too little attention to cane yield when managing irrigation during the dry-off period.

Measurement of plant extension rate and photosynthesis simultaneously can provide a good indication of the ripening response to factors such as water stress, temperature and chemical ripeners. Thus it may be possible to rapidly screen chemical ripeners for efficacy on a large range of genotypes and also to predict how long it would take to obtain an economic response to ripener application.

Recent efforts to simulate the observed responses of P, PER and SC within internodes will undoubtedly help to predict phenological, seasonal and management effects on SC and thereby assist with management options such as reduced irrigation or application of ripeners to improve SC and sucrose yield,

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CSIRO RESEARCH

Sunlight a critical factor on cane yield and CCS

This is another in the series of monthly columns from the CSIRO dealing with crop growth and productivity issues in the sugarcane industry. The article was written by Dr Geoff Inman-Bamber, a systems agronomist with the Davies Laboratory in Townsville, and Steve Attard, a researcher based in the Burdekin.

While sugarcane is efficient at converting the sun's energy into biomass and sucrose, lots of sunlight is needed for high yields and CCS.

Like practically all life on earth, sugarcane depends on energy captured from the sun through the process known as photosynthesis.

This is when plants and other organisms produce simple carbohydrates from carbon dioxide and hydrogen, using energy that chlorophyll or other cellular pigments absorb from the sun.

Sugarcane belongs to a group of crops (including maize and sorghum) that use the highly efficient form of photosynthesis known as the C4 pathway, in addition to the less efficient C3 pathway used by temperate crops.

In sugarcane, the C4 pathway predominates and allows it to retain more of the carbon dioxide (CO₂) fixed during day-time photosynthesis.

Visible light is used in photosynthesis to make sucrose in the leaf. It is then transported down the leaf to the top of the stem where it is used for growth, and later into the stalk, where it is stored if not required for growth.

Of all the sun's energy received by sugarcane, only 1% to 2% is used for photosynthesis. The rest is used to generate heat and for evaporation and transpiration (when the plant sweats to cool itself).

Plants need the heat for biochemical reactions. Stalk growth rate, for example, will increase by about 1.5



Research indicates that plant cane is most efficient at photosynthesis.

mm per day for each degree increase in daily minimum temperature above 8°C, provided water is not limited. Interestingly, photosynthesis itself is not affected by temperature to the same extent as stalk growth.

Excess energy not used for photosynthesis or heat is released in evaporation or transpiration. This is a nuisance because it takes a lot of precious water for the plant to soak up the sun's energy.

Radiation levels vary from year to year

Sugar yields are highest in the Burdekin, where solar radiation is highest and water abundant.

In the super-wet regions of Queensland, such as Tully and Babinda, yields can be poor because of low radiation, despite plenty of water. This is because of frequent cloudy weather.

Research supported by the Sugar Research and Development Corporation indicates that plant crops are most efficient at photosynthesis, making a maximum of about 1.8 gm of biomass (dry matter) per mega

joule (MJ) of radiation trapped by the leaves. Ratoon crops are only slightly less efficient at about 1.65 gm/MJ.

Average photosynthesis rates through the growth cycle are about 1.4 gm/MJ for plant crops and 1.2 gm/MJ for ratoon crops.

Annual levels of radiation in the Burdekin are about 7300 MJ per square metre (20 MJ per square metre per day), whereas Babinda only gets about 6200 MJ per square metre (17 MJ per square metre per day).

About 65% of this is caught by leaves of a 12-month ratoon crop, which should then yield about 57 tonnes of biomass per hectare. Of this biomass, a maximum of about 80% is in the stalk, about half of which is sucrose.

So the potential sucrose yield for a 12-month ratoon crop is about 23 tonnes per hectare in the Burdekin and only about 19 tonnes per hectare in the Babinda region, the difference due to levels of solar radiation.

Radiation levels vary greatly from year to year. The effect of this on yield can be seen most clearly in the Burdekin, where other factors such as water supply are usually adequate.

Cane yield for the 2000 season was low (104 tonnes per hectare) compared to the average for the preceding years 1995-1999 (123 tonnes per hectare). This was partly due to low sunlight during the period of most rapid growth.

The months of November 1999 and February and April 2000 were very cloudy and radiation received by the crop was between 20% and 26% lower than the average.

A similar situation occurred this year when radiation levels during April and May were up to 20% lower than the 10-year average.

Considering its high rates of photosynthesis, sugarcane is a good candidate for a biofuels industry. ■

Research

Factors affecting the optimal growth of your crop

By CSIRO researchers Steve Attard and Geoff Inman-Bamber

Everyone knows how fast things grow after rain and sugarcane is no exception. Your lawn may grow 10 mm in one day but sugarcane leaves can grow 110-130 mm in a day and up to 7 mm in a single hour. In hot and humid conditions, cane stalks elongate up to 40 mm a day, which is only a quarter the rate of leaves but still remarkable.

You can see the difference in growth rate between leaf and stalk by cutting the youngest four leaves at the same height and returning after a few hours to see how the newest leaves have extended beyond the older leaves.

This is because the newest leaves are pushed upwards by leaf and stalk cells that are multiplying and enlarging at the very tip of the stalk (called the meristem). Leaf plus stalk extension is known as plant extension or elongation.

Factors affecting growth rates: Leaf and stalk growth depends mostly on

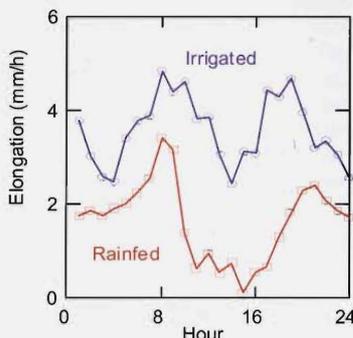


Figure 1: Daily pattern of plant elongation rate for an irrigated and rain-fed crop

temperature, sunlight and moisture. Elongation can be quite high at night if the temperature stays warm, but slows during cooler nights.

Elongation often increases in the morning as the temperature rises but then decreases again during the heat of the day because of water stress.

Sunlight only limits growth after several days of very dull weather.

Water stress – friend or foe? If you grow cane in a glasshouse where the humidity is always high, leaves will grow most rapidly at midday because of the higher temperatures.

However in the field, humidity is normally lowest at midday and plants suffer some water stress even if they are being irrigated.

In fact, this water stress is necessary to draw water through the plant much like the suction required to lift water up through a drinking straw.

Leaf growth then slows down because enlarging cells are temporarily under stress.

Irrigation and plant growth: Figure 1 shows a typical 24 hour pattern of plant extension for a crop that is well irrigated (blue) and one that is water stressed. In the irrigated crop, growth rates drop overnight, rises in the morning and falls again in the midday heat, before increasing again later in

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Research

the day as demand for water falls.

A similar pattern can be observed in the non-irrigated crop except that growth rates are much lower and growth practically stops in the afternoon. From this we can see that the greater the stress, the more growth occurs at night rather than during the day.

Optimising irrigation for maximum plant growth: In a recent case study, measurements of plant growth were used to assess the effectiveness of one grower's irrigation practice.

During the monitoring period, from January to mid April, the crop received a large number of irrigations and rainfall events and stalk elongation responded to each one, reaching about 28 mm/day between events.

During January the farmer irrigated frequently but insufficient water was being applied to fill the soil profile. As a result the soil water deficit widened and the crop experienced

increasing water stress and slowing growth rates.

A good rainfall in mid-February filled the profile and relieved the stress, but as the soil water deficit increased again, stalk growth rates slowed and almost stopped completely in late March, when the deficit reached 120 mm.

From late March, irrigations were scheduled more frequently so the crop

Computer program models crop growth

was never under water stress.

However by this time the weather had changed and the lower daily temperatures limited growth rates to below 15 mm/day.

By studying plant growth rates, irrigators can determine when to

irrigate their crop. In this paddock, a soil water deficit of about 50 mm would have been suitable.

WaterSense delivers paddock-by-paddock irrigation advice:

WaterSense is a computer program accessed via the Internet which simulates the growth of the crop and predicts when it should be irrigated to avoid a defined level of water stress.

Soil water deficit, rainfall and irrigation are depicted for each paddock and as a back-up, researchers measure leaf and stalk elongation to make sure that the program calculates the irrigation schedule accurately.

WaterSense was developed with funds from SRDC and CSIRO and will soon be tested and promoted. Funds are being sought to obtain supporting crop growth and soil data to make it accurate for a wide range of cropping conditions.

Contact Dr Geoff Inman-Bamber on 07 4753 8587. ■

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ERGON

RESEARCH

Tillering and canopy development – the inside story

This is another in the series of monthly columns from the CSIRO dealing with productivity issues in the sugarcane industry. This article was written by Geoff Inman-Bamber and Alan Garside

Does tillering and early canopy development affect crop yield? The simple answer to this physiology question is "yes", but the situation will determine whether the effect is positive or negative.

Early canopy development is largely dependent on the number of tillers a cane variety produces.

Varieties that tiller profusely can more rapidly form a closed canopy and intercept incoming radiation earlier in crop growth than varieties that are shy tillers. This early canopy closure might appear to be the avenue to higher yields.

However, the situation is not that simple. Cane yield comprises a combination of stalk number and stalk weight and, in general, where there are many stalks they will be small stalks.

Further, many of the tillers produced die-off, generally around the time when shoots start to become stalks. Although some shoot loss seems to be unavoidable, the degree of loss can be moderated by optimising growing conditions.

For example, research by the Sugar Yield Decline Joint Venture indicates that shoot/stalk retention can be enhanced under good growing conditions (good soil health, reduced compaction).

Practices such as reduced spacing (high-density planting) and increased water and nutrient inputs have been used to enhance canopy development, and they certainly do lead to additional tillers and stalks.

However, they will only lead to higher yields if the additional tillers and stalks make it through to harvest. If they die before harvest they may have used valuable water and nutrients, like weeds.

There are other considerations detracting from the importance of early canopy development.

In dry regions, practices that enhance early canopy development will

increase demand for water which may not be available. In high-yielding areas such as the Burdekin, the advantages of early canopy development may be lost because of increased lodging and ageing, which reduce growth. The ageing process is not well understood but is linked to reduced nitrogen content of leaves and stalk death.

Certainly, it is important to have adequate stalks to provide a high yield potential. However, heavy tillering and early canopy closure will only provide higher yields if there is the capacity to minimise their loss, convert them into stalks and adequately fill those stalks with sucrose.

There are many instances where that does not occur, so it is important that we understand the combination of factors that lead to high yield in sugarcane. Rapid early tillering and canopy closure certainly does not guarantee high yield. In fact in many circumstances it can provide the reverse.

We are increasing our understanding of these issues through further research projects on crop physiology, which are being supported by SRDC. ■

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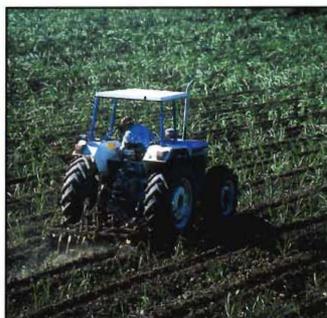
Why a July ratoon out-yields an October ratoon

By Geoff Inman-Bamber and Steve Attard,
CSIRO Sustainable Ecosystems

When we talk about ratoons we could be thinking about four important issues at the same time: the ratoon date, the crop class (as in first, second, third ratoon), the age of the ratoon crop and its date of harvest. All these will influence the yield and Commercial Cane Sugar (CCS) of the ratoon. Let's discuss them one by one.

Ratoon date: Critical experiments by Lisa McDonald of CSR confirmed what most growers know, that ratooning in November and December gives poor crops. There is no good physiological explanation of this, but we assume that heavy machinery on moist cane stools or possibly waterlogging suppresses subsequent growth. Should there be differences between crops ratooned between July and October? Basic crop physiology suggests that there will be differences but not as large as those arising from stool damage in moist soils.

With all other factors equal and no lack of water and nitrogen, an October ratoon will often out-yield a July ratoon because canopy development in July is much slower than in October



Cultivating ratoons.

and the July ratoon will waste more radiation even though both radiation and temperature are low in July. This effect will be accentuated when winters are cold. In Bundaberg for example, the canopy of a July ratoon may take four to five months to close, during which time a lot of radiation is wasted. The canopy of an October ratoon may be completed in two to three months and, although this is during a period of high radiation, less is wasted. The more radiation captured by the canopy the higher the yield will be unless other factors like water become limiting.

Crop class: We also have no good physiological explanation as to why

yields should decline with ratoon age as they do. Some farmers in Swaziland are harvesting crops ratooned more than 30 times without a decline in yield. With the right varieties and the right conditions (manual harvesting) sugarcane can ratoon indefinitely. An experiment in Mackay indicated a 13% decline in radiation use efficiency (photosynthesis) from the first to the second ratoon but we are not sure what causes this. Soil compaction by harvesters and other heavy machinery is a likely cause.

Age of the ratoon crop: There is often a pattern that growers follow when it comes to harvest date. March plant crop tends to be harvested around September, the following first ratoon in July and the following second ratoon in August. The plant crop benefits from a long growing period (about 18 months); the first ratoon crop suffers from a shorter growing season (about 10 months), while the second ratoon benefits from a 13-month growing season. The longer growing season will benefit the second ratoon crop through increased cane yield and higher CCS. Productivity of the first ratoon crop has suffered due to its lack of maturity.

Date of harvesting: CCS increases with age (maturity) but is also subject to marked seasonal variation. Dr McDonald's experiments indicated that CCS is dominated by seasonal effects around August and September and by age effects early in the year. Young crops can have high CCS in August and low CCS early in the season when CCS of old crops can be reasonably high.

Unlike annual grain crops which can be planted and harvested at the best time, cane growers have to harvest often under less than ideal conditions. Even if growers don't have much choice about when they harvest their ratoons, the above points should help to explain why some crops perform better than others. ■



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CSIRO unlocking the secret to higher CCS

By CSIRO's Dr Geoff Inman-Bamber and Dr Graham Bonnett

Sucrose, the main component of CCS, is stored in the sugarcane stalk only when it is not needed as a basic building block for new leaves and stalk tissue.

Making the most of this fact is one of the challenges of a major experiment at CSIRO Davies Laboratory in Townsville, funded by the Sugar Research and Development Corporation, with financial input also from CSIRO.

The research, with a combined value of \$1.5 million over four years, is for the first time in Australia integrating the molecular biology, physiology, breeding and agronomy of sugarcane to unlock the secret of optimising CCS.

Until now there has been a significant knowledge gap. Cane growers and scientists know that there are environmental and chemical factors that increase CCS.

In the past three decades progress in sugarcane research at all levels from the molecular to the crop has fallen well behind other major food and fibre crops, and little is known about the mechanisms for increasing CCS.

The SRDC-funded CSIRO research is addressing that shortfall in a cross-disciplinary series of experiments at the frontier of sugarcane knowledge.

It is known that when leaf and stalk growth is rapid, during hot and wet conditions, only a small amount of sucrose is stored.

In the winter/dry season, leaf and stalk growth slows. Provided soils are not too dry, photosynthesis will continue even after stalk growth stops, filling more of the stalk up with sucrose.

The project is testing a hypothesis based upon the source/sink idea. Photosynthesis is the main *source* of



Geoff Inman-Bamber (back) and Alan Garside inside the Tall Plant Facility at the CSIRO's Davies Laboratory ... major experiment.

dry matter, including sucrose, and the various plant components (including stored sucrose, as well as leaf growth) are the *sinks* to which the products of photosynthesis flow.

These sinks compete with each other for the limited amount of material produced by photosynthesis.

Understanding exactly how the plant deploys the products of photosynthesis under various conditions may give scientists the means to control the process.

Water stress is often more reliable than ripeners

A major part of this work has been conducted in the Tall Plant Facility at the Davies Lab in Townsville in strictly controlled environments.

While the results of the experiment, conducted earlier this year, are not in yet, preliminary indications are that it has succeeded in producing greatly increased sucrose content (up by 30%), by reducing the plant extension rate (PER) over about 20 days.

In essence, this means that the plant stores sucrose rather than use it for energy to grow more stalk and leaves

if it is subjected to water stress (under-watering). This water stress has to be at the right level not to affect photosynthesis, but enough to prevent the plant from growing excessively.

These data will be important in finding ways to harness the physical processes to increase sucrose content and possibly yield as well.

On the farm, clever irrigation regimes and chemical ripeners can help to limit expansive growth without reducing photosynthesis too much and so we can get the benefits of high photosynthesis during the sunny winter months without producing much more cane, which should have been produced mainly in summer.

Controlled water stress by drying off is often more reliable than ripeners, which react differently, depending on the variety and climatic conditions.

Drying off and ripeners can't be used together because they use the differential responses of photosynthesis and expansive growth to divert sucrose to the stalk.

A future column will outline the results from the water stress experiment. ■

RESEARCH

Using less water to boost cane and sugar yield

This is another in the series of monthly columns from the CSIRO dealing with crop growth and productivity issues in the sugarcane industry. This article was written by Geoff Inman-Bamber, a systems agronomist with the Davies Laboratory in Townsville, and Steve Attard, a researcher based in the Burdekin.

Efficient use of water is fundamental to increasing profits from sugarcane production.

About 60% of sugar produced from cane in Australia requires some form of irrigation, so managing this resource well makes a big difference to the health of individual farms and the industry.

Particularly in places where dry conditions can prevail, crop water requirements need to be gauged accurately.

The goal is to use less water, but increase the cane and sugar yield. This will not only benefit the bottom line, it will also mean less off-site impacts such as water run-off.

The key is to plan ahead and use water at the best time for the crop. This requires knowledge of how water

stress affects crop growth.

Sugarcane seldom uses more than 8 mm of water a day as there are physical limits set by energy entering and leaving the system.

The response of sugarcane to irrigation, or lack of it, can be classified into three phases: early canopy development, late canopy development and stalk elongation and the maturing phase.

In the first phase, water loss through evaporation from the soil is a problem, but mulching with a trash blanket can reduce evaporation by 50%. Limiting irrigation to a minimum during this time will also help.

Following an initial irrigation after planting, sugarcane can withstand several months of water stress without cane and sucrose yield being affected. Research in the Burdekin has demonstrated the resilience of sugarcane to water stress during early expansive growth.

In late canopy development and stalk elongation, water stress can harm cane and sucrose yields. Research funded by SRDC in the Herbert on Roy Pace's farm shows responses to irrigation during this phase can be high (up to 27 tonnes of cane/ML).

Irrigating when stalk elongation rates fall to 50% of potential is recommended to ensure water does not limit biomass or sucrose yield. In our experiments, however, biomass was not reduced in water-limited situations until stalk elongation dropped to less than 30% of the rate in well-irrigated crops.

In the maturing phase, research shows extended drying-off enhances sucrose content which offsets losses in cane yield. The highest economic benefit from drying-off is likely to occur when water stress reduces cane yield by between 4% to 8%.

It's the uncertainty of rainfall and the wide range of crop growth stages on farms which complicates irrigation management. Sometimes it may be best to use limited water during the early development phase and, in other cases, it may be best to use irrigation later.

In general, however, irrigation applied in the dry months (September to December) leading up to the wet season has the greatest benefit.

CSIRO's Davies Laboratory is now trialling a web tool called WaterSense for calculating optimum scheduling of irrigation with limited water supply and uncertain rainfall. ■

INDUSTRY NEWS

Biofuels partnership

DuPont and BP have created a partnership to develop, produce and market a next generation of biofuels to help meet increasing global demand for renewable transportation fuels. DuPont and BP have been working together since 2003 to develop advanced biofuels with properties that can help overcome the limitations of existing biofuels. DuPont and BP are working with British Sugar, a subsidiary of Associated British Foods plc, to

convert the country's first ethanol fermentation facility to produce biobutanol. Additional global capacity will be introduced as market conditions dictate. A feasibility study in conjunction with British Sugar is already under way to look at constructing larger facilities in the UK. The companies' joint strategy is to deliver advanced biofuels that will provide improved options for expanding energy supplies and accelerate the move to renewable transportation fuels, which lower overall greenhouse gas emissions. The

first product to market will be biobutanol. ■

Sugar tax under review

Federal Agriculture Minister Peter McGauran will review the 3c/kg sugar tax on food and beverage manufacturers in Australia. The tax was imposed to provide funding for the sugar industry reconstruction during the world sugar price slump. Some analysts are now saying that the world resurgence in sugar prices make the tax unnecessary. ■

Clever irrigation can help roots go deeper

This is another in the series from the CSIRO about crop growth and productivity. This article was written by Dr Geoff Inman-Bamber, a systems agronomist with the Davies Laboratory in Townsville, and Steve Attard, a researcher based in the Burdekin.

The development of the root system in sugarcane starts soon after planting.

The first roots formed are sett roots, which emerge from the planted cane billet. The next are shoot roots, which start from the base of the new shoot about seven days after planting and grow rapidly at about 40 mm a day in sandy soils and 28 mm a day in heavy clays.

Scientists have observed the rate of descent of the entire root system to be 20-30 mm a day to a depth of 1.6 m for rain-fed crops; 17 mm a day in irrigated crops to 1 m and 6 mm a day in crops between 1 m and 1.6 m.

Root growth responds strongly to the soil environment. Soil compaction reduces root growth and causes roots

to thicken, with reduced branching.

Root mass can shrink in poorly structured heavy clays subject to water-logging to a fifth of what it should be.

Above-ground yield will be limited by poor root growth because of reduced capacity to use available water and nutrients for production.

The size and distribution of the plant's root system will be affected by the distribution and availability of soil water.

In one experiment, 20% of the root mass was deeper than 1 m when irrigation was applied each week, compared to 35% when irrigation was applied every three weeks.

A CSIRO experiment on deep-red volcanic soil on Graham Webb's farm



Graham Webb ... hosted an experiment on deep-red volcanic soil showing how roots were active at 2.8 m when surface layers dried out, but were inactive when it rained.

at Childers showed how roots were active at 2.8 m when the surface layers dried out, but were inactive when it rained again.

Other experiments with nitrogen fertiliser by Peter Thorburn of CSIRO also demonstrated the adaptability of the root system.

Where no fertiliser was applied to a plant crop, nitrogen was removed from soil in both row and inter-row positions and from a depth of 1.5 m.

Where fertiliser was applied, there was no uptake from the interrow and little from 1.5 m depth.

These experiments show that the root system of sugarcane can explore extensively the depth and breadth of the soil, absorbing water and nutrients when they are not available near the surface.

In the past, it was thought the root system died after harvest.

Sugarcane can extract water and nutrients from considerable depths and can make use of water upflow from water tables.

Shallow roots are caused more by soil constraints than intrinsic root system characteristics.

Research last year by Mike Bell (Department of Primary Industries and Fisheries) and Alan Garside (BSES), of the Sugar Yield Decline Joint Venture, indicated that better knowledge of root system responses to the soil can address constraints on productivity and underpin development of farming systems which were more successful at sustaining soil health.

More roots at depth can be obtained by irrigating only when necessary.

A reasonable amount of water (from 30 mm to 50 mm) applied after planting or ratooning should help roots to reach a depth of 1 m within about seven weeks. ■



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