

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).

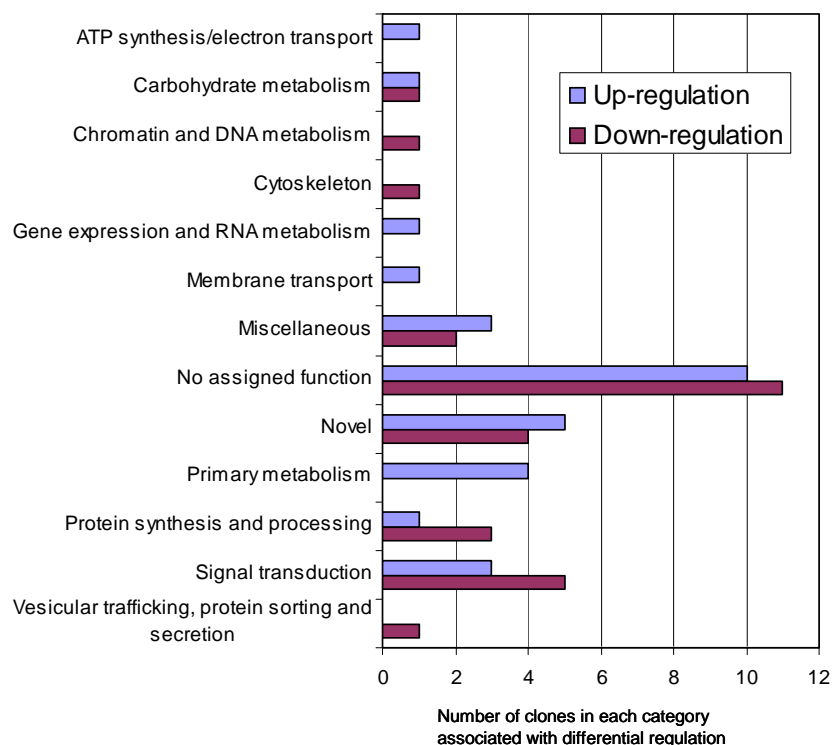


Figure 1. Classification of genes that were up-regulated or down-regulated in high CCS progeny into functional groups. The functional groups are as described in Casu *et al.* (2001, 2003, 2004). Reprinted from *Field Crops Res*, 92, 2-3, Casu *et al.* (2005), with permission from Elsevier

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.

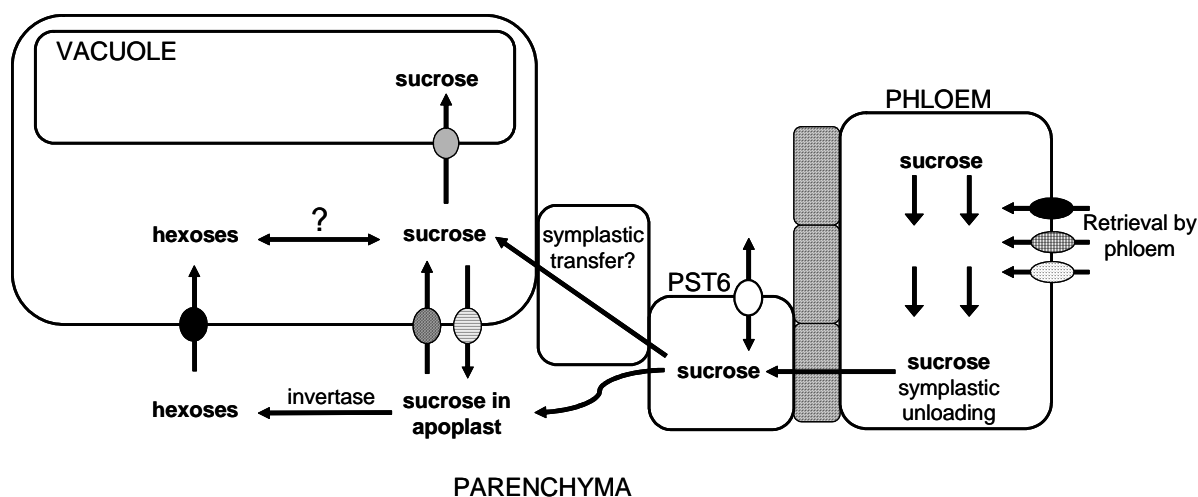


Figure 2. Proposed model of sucrose transport between phloem and parenchyma vacuoles. PST6 = putative sugar transporter no. 6. Reprinted from *Field Crops Res* 92: 2-3 and Rae *et al.* (2005), with permission from Elsevier.

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 $^{13}\text{CO}_2$ 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.

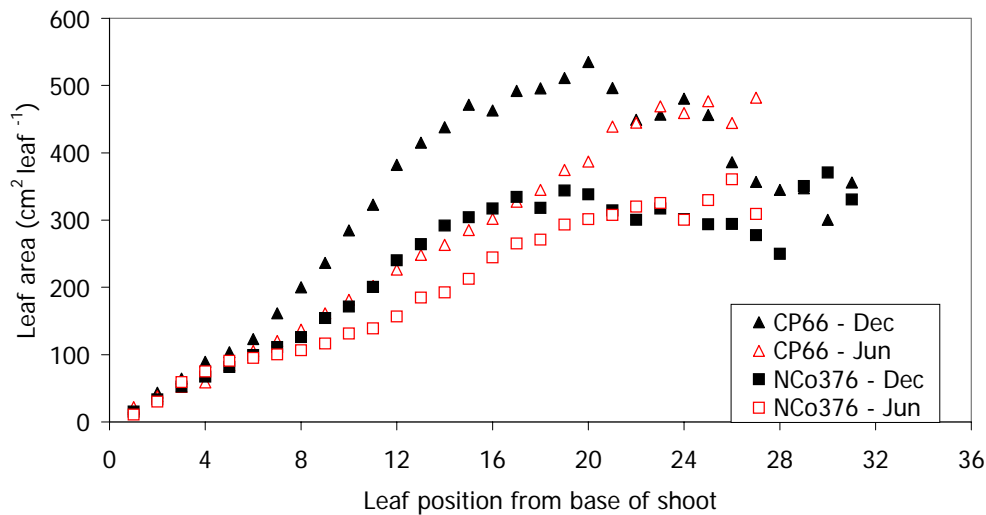


Figure 3. Mean area of successive leaves (bottom to top) on stalks of NCo376 and CP66/1043, ratooned in June or December at Mount Edgecombe. Reprinted from *Field Crops Res* 92: 2-3 and Singels *et al.* (2005a), with permission from Elsevier.

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.

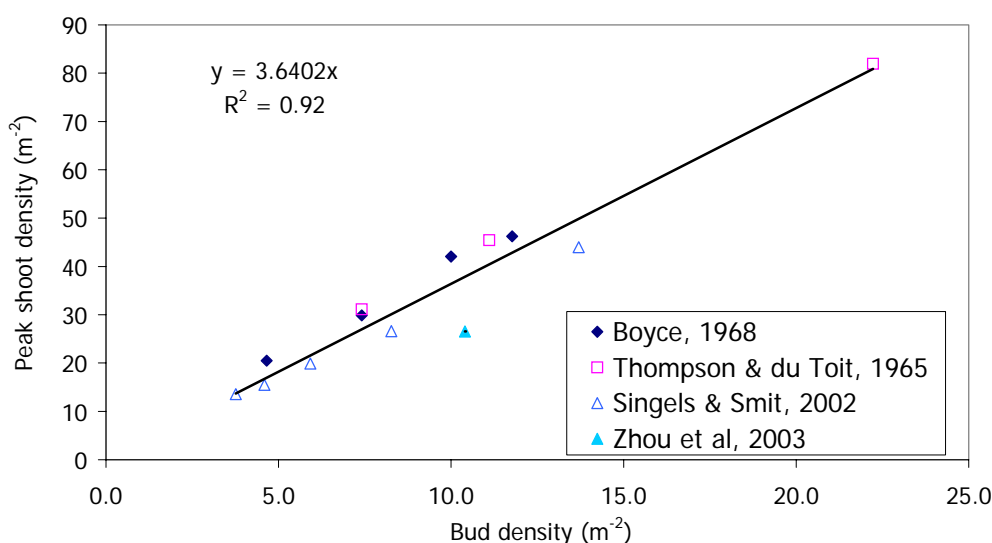


Figure 4. Measured peak shoot density as a function of the initial bud density for various experiments. Bud density was estimated from the amount of seedcane planted (one bud per 10 cm) for Thompson and du Toit (1965) and Singels and Smit (2002). Zhou et al. (2003) and Boyce (1968) measured bud density. Reprinted from *Field Crops Res* 92: 2-3 and 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 $>32^{\circ}\text{C}$ 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/m^2 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).

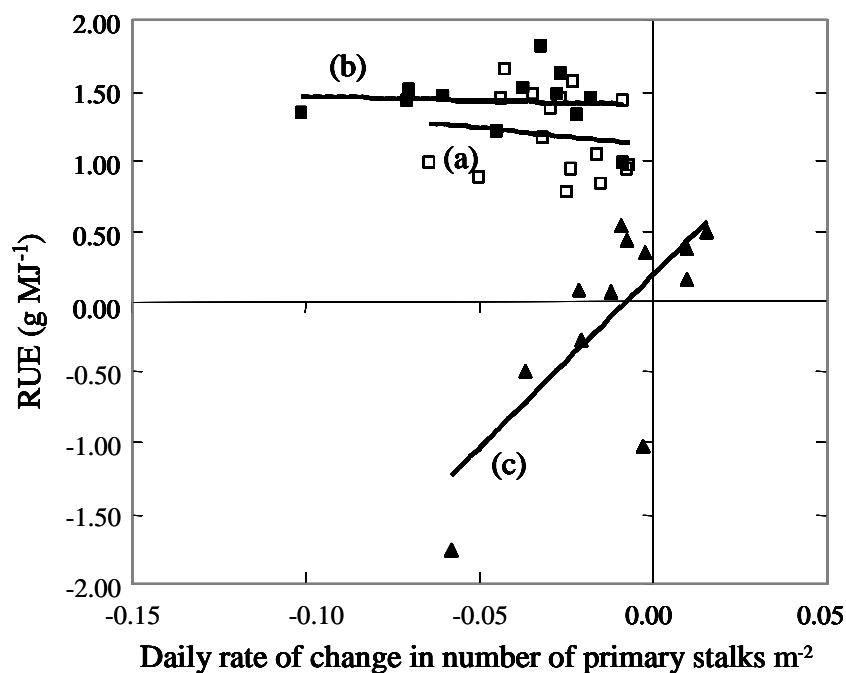


Figure 5. The relationship between the daily change in the number of stalks/ m^2 from the first count taken at approximately 80 days after crop start and (a) maximum RUE in treatments where no RUE decline was observed (\square), (b) maximum RUE before it declined (\blacksquare), and (c) average RUE after the decline (\blacktriangle). 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 and 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.

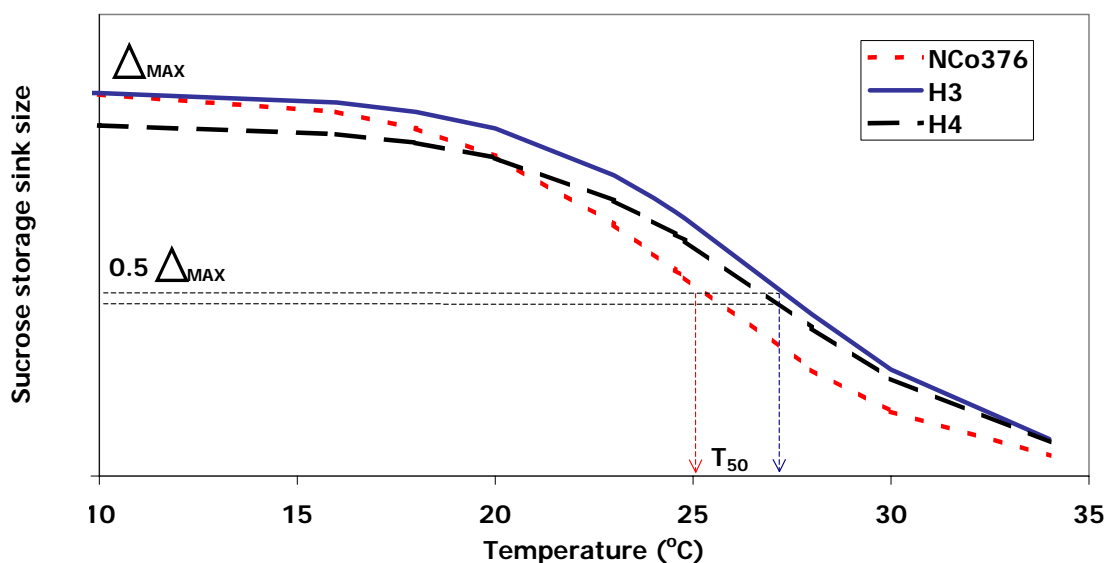


Figure 6. The effect of temperature on the capacity of three cultivars to store sucrose in the stalk. These functions are determined by the parameters Δ_{MAX} and T_{50} . Reprinted from *Field Crops Res* 92: 2-3 and Singels *et al.* (2005b), with permission from Elsevier.

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 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

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

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.

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