

THE EFFECT OF HEXOSE UPON POL, BRIX AND CALCULATED CCS IN SUGARCANE: A POTENTIAL FOR NEGATIVE POL BIAS IN JUICE FROM ACTIVELY GROWING CANE

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ABSTRACT

Commercial cane sugar (CCS) is calculated from Brix, Pol and fiber content of cane. CCS estimates the level of extractable sucrose minus a negative weighting based on the level of impurities at harvest and is the basis of payment to the farmer in the Australian sugar industry. Here the effect of physiological levels of hexose impurities, glucose and fructose, upon Pol and CCS using laboratory prepared sugar solutions are examined. Unlike previous reports, we have quantified the negative effect of biological levels of hexoses upon Pol and determined that, under certain conditions, it may be sufficient to affect CCS. The negative effect on Pol and CCS was linear with increasing hexose concentrations, affected by the glucose/fructose ratio and independent of sucrose concentration. The potential for a hexose induced Pol is therefore greatest in actively growing cane when the hexose concentrations are high and the levels of glucose and fructose are similar. A review of field CCS data from very immature, actively growing cane supported the proposal of a potential hexose induced Pol bias as large extremes in CCS were recorded across clones with similar Brix. The consequence of identifying a potential hexose induced negative bias of Pol and CCS on research examining sugar accumulation in developing sugarcane is discussed.

Key words: Pol bias, hexose, CCS, Brix

INTRODUCTION

CCS is the basis of payment in the Australian sugarcane industry and some other parts of the world. It is not a direct measure of sucrose content but rather an estimate of total sugar (%) (Brix) adjusted for purity (Pol) and stalk fiber content. The Brix value estimates total soluble sugars whereas Pol indicates sugar composition and is determined using a polarimeter. A polarimeter measures the rotation of light passing through a solution. The degree of polarisation is dependent upon the specific rotation of a compound and its concentration.

The CCS formula was developed by Dr. Kottman in 1888 and was derived from mill records (King *et al.*, 1965), using commercially harvested cane. As impurities reduce the recovery of sucrose from cane juice during the milling process, the CCS formula has a strong weighting against impurities. Impurities can be ash, dirt, sugars other than sucrose etc. which effect the crystallisation and therefore the recovery of sucrose (King *et al.*, 1965).

Sucrose is a disaccharide formed from two hexoses, glucose and fructose. Glucose and fructose are the predominant hexoses in sugarcane (consequently total hexose content reflects the total of these sugars) and are components of the impurity fraction. As cane matures, the stalk sucrose:hexose ratio increases.

Brix and Pol values determined from sugarcane juice are important parameters in calculating CCS. The specific rotation of glucose, fructose and sucrose is + 52.7, - 89.5 and + 66.5 respectively at 20 °C (CRC Handbook, 1971-72). In a solution containing a mixture of glucose, fructose and sucrose, the degree of polarisation is the net rotation of all three sugars. The negative specific rotation of fructose far exceeds the positive specific rotation of glucose (1.7 fold) and at glucose/fructose ratios of less than 1.7 the negative polarisation of the hexoses will detract from the positive rotation of sucrose resulting in lower Pol than if sucrose was present alone. Similarly a glucose:fructose ratio exceeding 1.7 will increase Pol over and above the Pol resulting from sucrose.

Although Pol is not a direct measure of sucrose content, Pol readings obtained in the sugar industry that give an estimate of sucrose content that disagree greatly with the actual sucrose content have been referred to as 'false Pol' or 'Pol bias' (Chen *et al.*, 1971; Irvine 1985). Previous reports of false higher Pol readings have attributed it to a high glucose/fructose ratio (Chen *et al.*, 1971), or alternatively, to an unknown impurity (Irvine, 1985).

DeStefano (1985) and Robertson *et al.*, (1996) both reported that a hexose induced negative bias in Pol may occur but that it was likely to be minimal due to low hexose levels and glucose/hexose ratios greater than 1.0. As Pol is strongly correlated to CCS, any Pol bias will also adversely affect CCS. However the effect of hexose on CCS is more complex as it will also influence another parameter used to calculate CCS, namely Brix. An addition of 1% w/v sugar to a sucrose solution will increase Brix by 1 unit. To obtain a positive bias in CCS from the sugar impurity it has to have a specific rotation greater than sucrose. In contrast, to have a negative bias in CCS the sugar impurity must have a specific rotation less than sucrose (< 66.5).

In the case of the hexoses, glucose and fructose, the negative effect on CCS is due to the high negative specific rotation of fructose exceeding the positive specific rotation of glucose and the increase in Brix. In very immature cane, the sucrose content is low and the hexoses high. Under these conditions, Pol will be low relative to Brix resulting in extremely low, possibly negative CCS.

Although the qualitative effect of hexose concentrations upon Pol and CCS are well described, they may not have been accurately quantified. Robertson *et al.*, (1996) examined the effects of crop age and nitrogen supply on the accumulation of reducing sugars in several sugarcane varieties. They reported higher levels of hexoses present in immature cane and elevated levels in cane supplied with high levels of nitrogen but determined that the hexose level had little impact on the measurement of Pol.

A simple experiment was devised to quantify the effect of high, but biological levels of hexose, upon Pol and calculated CCS. Brix and Pol measurements were performed on standard sugar solutions with varying sucrose and hexose concentrations and CCS calculated using an assumed fiber content of 13%. The findings from this experiment led to the detailed examination of previous reports of Pol bias and field CCS data of immature canes, which have high hexose concentrations.

MATERIALS AND METHODS

Standard sugar solutions

Standard solutions (250 mL) were prepared at three sucrose concentrations (5, 10 and 20 % w/v) with varying hexose concentrations (0.0, 0.625, 1.25, 2.5 and 5% w/v), using AR grade sugars (Sigma[®], Sigma Chemical Co Ltd. Missouri, USA). The hexose levels were prepared from equivalent amounts of glucose and fructose at concentrations to reflect biological levels for field grown cane.

Sugarcane growth experiment at four temperatures in the CSIRO Constant Environment Facility

An experiment conducted in the Controlled Environment Facility (CEF) at CSIRO Plant Industry, St Lucia, examined two commercial sugarcane varieties, Q117 and Q138, grown under constant conditions for two months then subjected to one of four different ambient temperature regimes (14, 18, 22, 26° C) for 120 days. The plant material was germinated and grown as described by Campbell et al., (1996). Plants were harvested prior to the imposition of the treatments, then at monthly intervals for biomass, carbon and sugar partitioning. The juice was pressed out of each internode and immediately frozen on dry ice.

Field CCS data

Early season CCS was determined on a replicated field trial of a sugarcane population at the CSR Kalamia Mill, Ayr. The site consisted of 230 individuals replicated in 4 blocks. Urea was applied as fertilizer at 120 kg/ha to the plant crop.

Brix and Pol measurements

i) Standard Sugar solutions

Both Brix and Pol measurements were recorded for each standard sugar solution. Brix measurements were conducted using a hand held Brix meter (model N-1, Atago Co., Ltd, Japan). The meter was zeroed using Milli Q water prior to obtaining a Brix measurement for each sugar solution.

Pol was analysed using a polarimeter (Quartz wedge Polarimeter with a standard Polaris Pol tube (200 mm), 589nm wavelength; Schmidt and Haensch GmbH and Co, Berlin, Germany) at Rocky Point Mill, Rocky Point, Queensland. The instrument was calibrated to zero and 100 units with Milli Q water and 26% (w/v) sucrose respectively in accordance with standard industry practice. Prior to recording Pol, the Pol tube was rinsed with a large volume (200 mL) of sample.

ii) Cane juice samples

Measurements of Brix and Pol from field harvested cane were conducted at CSR Kalamia Mill, Ayr, as previously described (Jackson and Morgan, in press).

CCS calculations

CCS is derived from a function of Brix in juice, Pol in juice, and cane fibre content. CCS was calculated using the CCS formulae detailed below. It is similar to the formula published by Muchow *et al.*, (1993), the exceptions are that T^2 in our formula replaces $T/2$ in theirs. Presumably the error arose in the Muchow *et al.*, manuscript during the publication process. The +200 at the end of the formula takes off 2 units of CCS to make a small mill juice sample equivalent to the commercial mill first expressed juice. For the standard solutions CCS was calculated from the Brix and Pol measurements using an assumed fibre figure of 13%.

CCS formulae (Excel ® spreadsheet): $CCS = (((39 * B * (95 - C)) / (99.82 + (0.415 * A))) - (0.5 * (A + ((0.00137 - (0.00003 * A)) * T^2) + ((0.00172 * (A - 0.0044)) * T) - (0.0224 * A) - 0.46) * (97 - C) + 200)) / 100$

Where A = Brix; B = Pol; C = Fiber; T = temperature and is set at 20°C in the calculations in the paper.

Sugar analysis of cane juice

The sugar content of cane juice samples was determined by HPAE-PAD (Papageorgiou *et al.*, 1997) or HPLC (Campbell *et al.*, 1999) analysis. Glucose, fructose and sucrose calibration curves ($R^2 \geq 0.998$) were produced from AR grade sugars (Sigma®).

RESULTS AND DISCUSSION

Brix and Pol measurements using standard solutions

For all solutions combined, the measurement of Brix correlated strongly ($R^2 = 0.9995$) (Fig. 1) with the percentage of total soluble sugars. However Pol (Fig. 2), decreased linearly with increasing hexose (equal concentration of glucose and fructose) and this effect was independent of sucrose concentration. Pol decreased by approximately 6.6 units with the addition of 5 % hexose.

The reduction in Pol under higher hexose concentration is due to the negative specific rotation of fructose exceeding the positive specific rotation of glucose. The loss in Pol is dependant upon the ratio of glucose to fructose and the hexose concentration. As glucose/fructose ratios approach 1.7, the negative rotation due to the hexoses will be reduced.

Hexose and sucrose concentrations in juice and stem samples

The major factors influencing Pol bias are the sugar concentrations and their relative ratios. Whilst the hexoses commonly are less than 5% of the total stalk sugars at harvest of mature cane, they can constitute a greater proportion at earlier ages of harvest (Robertson *et al.*, 1996). Hexose concentration in juice extracted from immature tissue is also high and may exceed 3% w/v. (Albertson *et al.*, unpublished data). Therefore under these conditions the magnitude of the Pol bias will depend on the ratio of glucose/fructose. Irvine (1985) and Robertson *et al.*, (1996) reported a ratio of glucose/fructose in several varieties ranging from 1.24 to 1.8.

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An experiment conducted in the CEF at CSIRO Plant Industry, St Lucia, examined two commercial sugarcane varieties Q117 and Q138, the same varieties used by Robertson *et al.*, (1996), grown at a range of temperatures. The average glucose/fructose ratios of internode juice across all temperatures was between 1.0 – 1.55. With increasing maturity the lower internodes reached higher sucrose concentrations and glucose/fructose ratios with very few individual measurements exceeding 1.7 (Fig. 3). This range in glucose/fructose ratio has also been recorded in our laboratory using the HPAE-PAD for juice from many varieties and tissue of differing ages. The ratio was always below 1.7 and therefore the negative specific rotation of fructose exceeded the positive specific rotation of glucose. However the hexose levels are very low in mature internodes and therefore the hexose induced Pol bias is likely to be negligible.

The higher glucose/fructose ratios observed by Robertson *et al.*, (1996) for the same two commercial varieties may be due to differing stalk maturity. The earliest harvest of material by Robertson *et al.*, (1996) was at 153 days after planting (DAP) whereas we examined the sugar content in cane as young as 85 DAP (immature plants described in Fig. 3).

The high glucose/fructose ratios in the two commercial canes may not represent the ratios present in diverse genetic material. In our laboratory we have recorded glucose/fructose ratios of approx. 1.0 in juice from sugarcane F1 populations (initial cross between two sugarcane varieties) and from a large collection of *S. officinarum* at various stages of maturity. Unlike the commercial canes that have already undergone a process of selection, the clones from a breeding population and the *S. officinarum* could have great variation in glucose/fructose ratios and hexose concentration. Therefore the potential for clones in breeding programs to have a glucose/fructose ratio of approx. 1.0 exists. Consequently the chance of a negative Pol bias is increased, particularly if selection is performed when the cane is immature.

Pol bias: Estimating the effect of hexose on Pol

Robertson *et al.*, (1996) estimated Pol bias on a g/g basis, however this does not indicate the change in Pol as recorded by the polarimeter. The polarimeter is scaled linearly from zero to 100 units using water and 26% sucrose respectively. Consequently, 1% sucrose is equivalent to 0.01g/g or 3.846 units Pol, therefore the Pol bias estimates of approx. – 0.003 to –0.007 g/g in immature tissue by Robertson *et al.*, (1996) could alter Pol by several units (2.69). Therefore in very immature cane when Pol is extremely low (< 30), this bias of Pol could be greater than the maximum of 6% Pol estimated by Robertson *et al.*, (1996) in mature cane and much higher if the glucose/fructose ratios approach 1.0.

Estimating the effect of hexose on CCS using standard solutions

The effect of hexoses upon CCS was estimated using Brix and Pol measurements from the standard sugars and an assumed fiber content of 13%. As described previously, the standard sugar solutions contained equal concentrations of glucose and fructose, conditions more similar to immature tissue. For any level of sucrose, the addition of 1% hexose slightly increases Brix and decreases Pol, however it has a much larger negative effect on CCS (Fig. 4). Robertson *et al.*, (1996) reported that CCS estimates did not agree with HPLC

measurements of sucrose at concentrations below 10% or greater than 15%. At sucrose concentrations below 10%, CCS underestimated sucrose content whereas at sucrose concentrations greater than 15%, CCS overestimated sucrose content. They concluded from low Pol bias values that the hexoses were not responsible for the variation between CCS and HPLC estimates. However, Robertson *et al.*, (1996) did not account for the effect of hexoses in reducing the estimate of sucrose and overestimating the level of impurities. The CCS formula can be simplified as described by BSES (1991),

$$\text{CCS} = \text{Pol in cane} - (\text{impurities in cane}/2)$$

where impurities in cane = Brix in cane – Pol in cane.

Having hexose present in a glucose to fructose ratio less than 1.7, as demonstrated lowers Pol and therefore underestimates the sucrose content. In the CCS formula this has the effect of overestimating impurities (Brix-Pol) and underestimating sucrose in the 'Pol in cane' term. Robertson *et al.*, (1996) reported hexose levels of >3% in juice from immature cane. Through overestimating impurities and underestimating sucrose content this would give rise to lower CCS estimates of sucrose content relative to HPLC measurements.

Indirect field evidence supporting a possible hexose effect on CCS in immature cane

Unlike previous reports on hexose induced Pol bias in commercial cane varieties, we have examined Brix and CCS measurements conducted at an early stage of growth (6 months) in an F1 sugarcane population. Although the CCS estimates are low in immature cane, a large range in CCS, up to 4 units, was observed between clones with similar Brix (Fig. 5). A similar level of variation in CCS was observed using standard sugar solutions with a constant Brix (Table 1). Therefore the variation in CCS observed in the immature F1 population could be due to variation in the level of hexose.

Sugarcane clones with a high sucrose and low hexose level very early in the season, will presumably exhibit a high CCS. In contrast, those with both high sucrose and high hexose levels exhibit a very high Brix but relatively low Pol, and therefore a much lower CCS. However, measuring CCS alone and not sucrose content directly will not yield information about the relative sucrose accumulating abilities of the different genotypes. This is particularly important in experimental programs aimed at understanding sucrose accumulation.

Potential consequences of hexoses affecting CCS as a measure of sucrose content in immature cane

Although CCS is relatively easy to measure and is appropriate for estimating recoverable sucrose from harvestable cane at the mill, it may not provide an accurate measure of sucrose content or sugar composition during early stalk development. As only a fraction of photosynthate is partitioned towards sucrose storage, the process of accumulating high levels of sucrose at maturity occurs over a long period of time and is initiated at an early stage of plant growth. It is important to accurately determine sucrose concentration and sugar composition of cane at all stages of plant development if the biochemical process underpinning sucrose accumulation is to be unraveled, ultimately for the long term benefit of the sugar industry.

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Robertson *et al.*, (1996) reported higher hexose concentrations in commercial varieties grown under high rates of nitrogen application compared to those without nitrogen. This indicates that the hexose level may also be affected by farming practices. The potential for hexoses to induce Pol bias, as detailed above, suggests that CCS may not accurately reflect the sucrose content or sugar composition in immature cane. Consequently if it is the only measure of sucrose used, particularly in experiments, it will obfuscate understanding of the processes leading to high sucrose accumulation.

CONCLUSION

We have shown empirically that physiological levels of hexose can induce a measurable change in Pol, which has been referred to as Pol bias. This contradicts the findings of DeStefano (1985) and Robertson *et al.*, (1996) who reported that the hexose concentrations and the ratio of fructose to glucose were insufficient to affect Pol. In the case of Robertson *et al.*, (1996) they calculated Pol bias but did not relate it to units of Pol before interpreting its effect on CCS. The conflicting reports could be due to underestimating the potential for Pol bias by examining commercial canes, which have already undergone selection for high CCS. In contrast, a diverse source of germplasm is likely to vary greatly in sucrose and hexose concentrations; glucose/fructose ratios and consequently Pol bias.

We also report that a hexose induced Pol bias can negatively affect CCS. This negative effect on CCS is greatest under high hexose concentration as occurs in immature cane, which is also low in sucrose. Therefore at low sucrose and high hexose levels, CCS estimates of sucrose content may be underestimated compared to HPLC measurements, as reported by Robertson *et al.*, (1996).

The potential for a hexose induced negative bias in Pol of juice from very immature cane exists and can affect CCS. Therefore research examining the biochemical processes involved in sucrose accumulation in developing cane should measure the stalk sucrose content using quantitative analytical techniques rather than the CCS formula.

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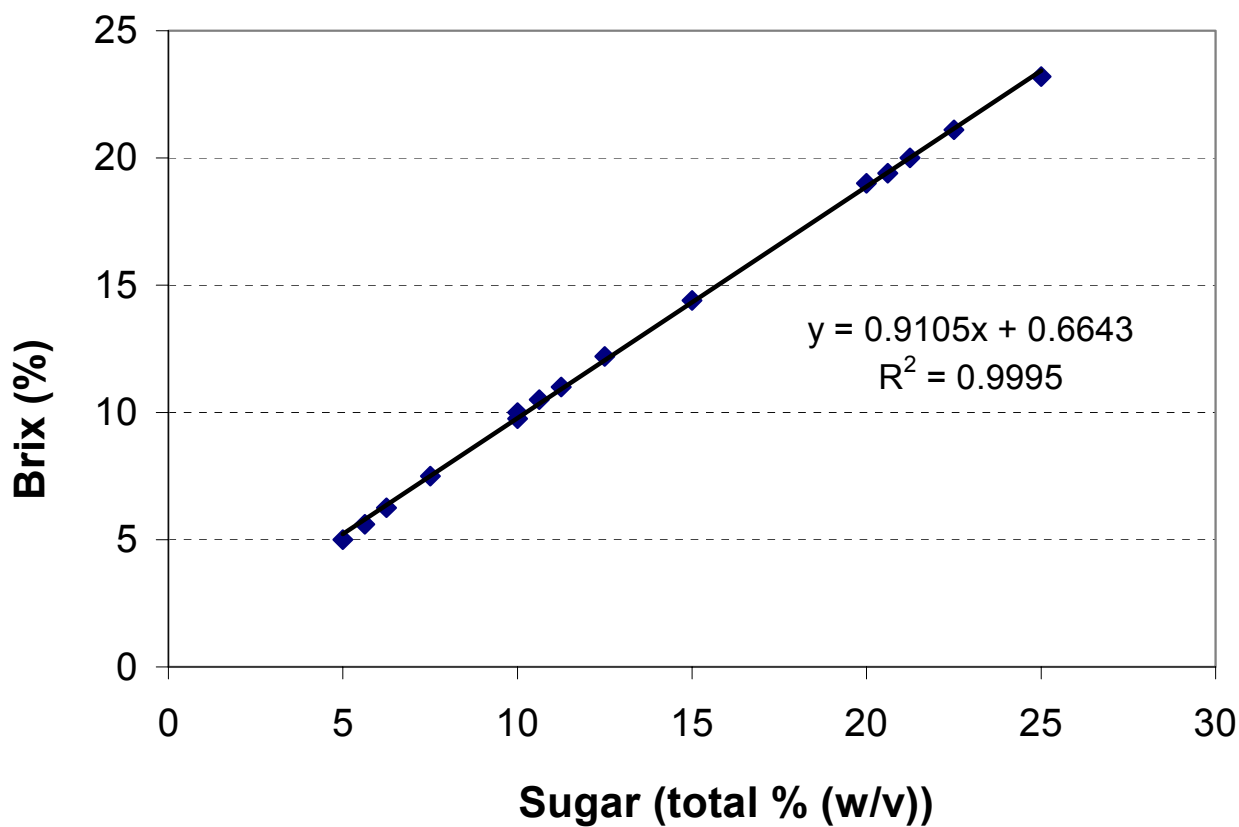


Figure 1. Refractometer readings of standard sugar solutions. Total sugar (%) is the combination of sucrose (%) and hexoses (%). Results are the mean \pm SE of three replicates.

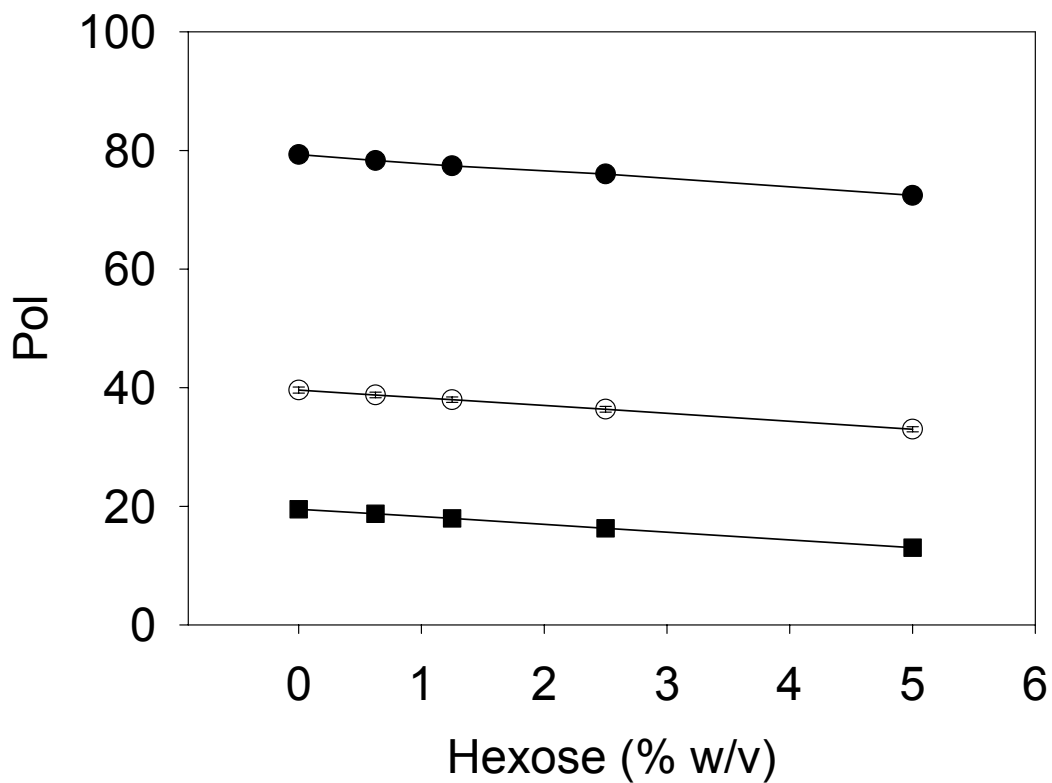


Figure 2. The effect of increasing hexose (%) on Pol at three sucrose concentrations. Sucrose concentrations were: 5% (■), 10% (○) and 20% (●) w/v. Results are the mean \pm SE of three replicates.

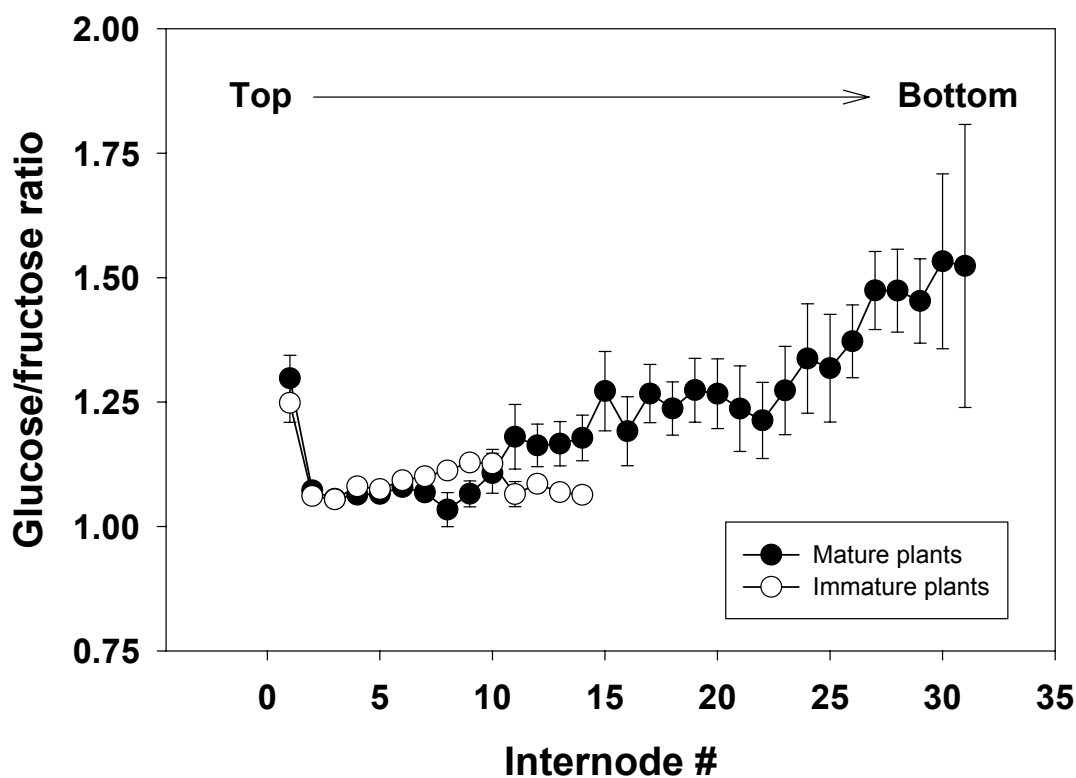


Figure 3. Glucose/fructose ratios in immature and mature internodes of two commercial sugarcane varieties (Q117 and Q138) grown in the Constant Environment Facility, CSIRO Plant Industry, Brisbane. Results are the mean \pm SE of a total of eight and 12 replicates per variety for mature and immature plants, respectively.

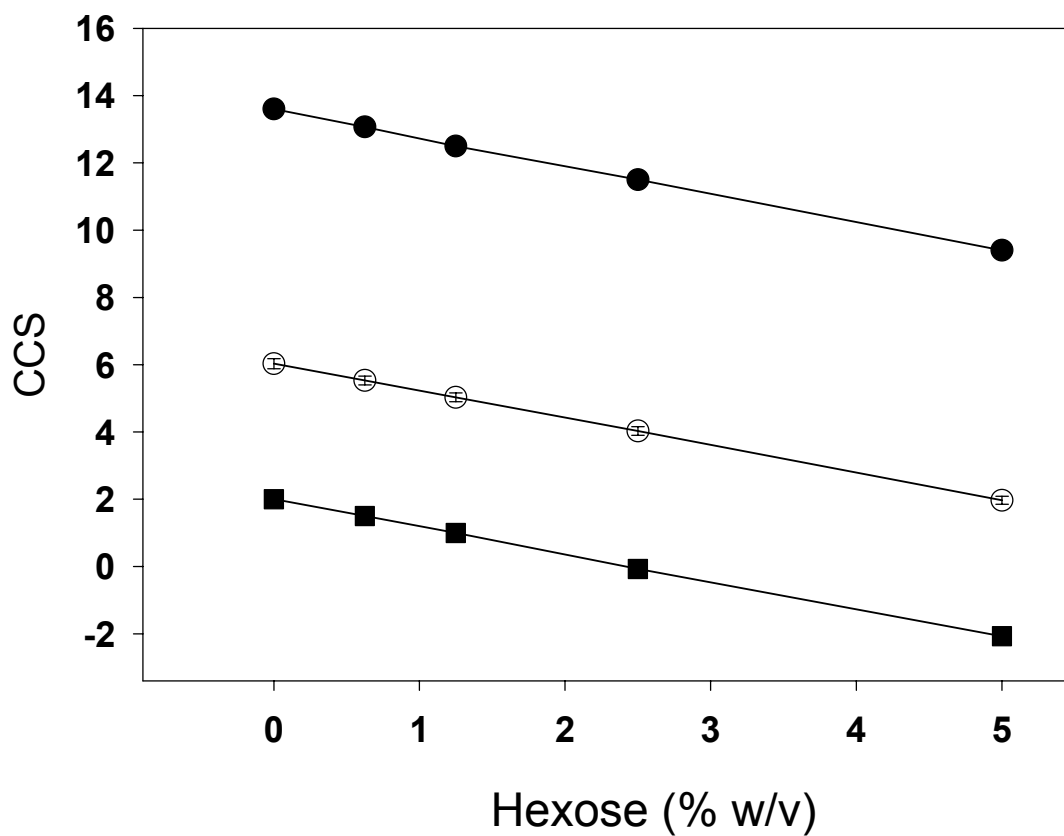


Figure 4. The effect of hexose (%) on CCS at three different sucrose concentrations. Sucrose concentrations were: 5% (■), 10% (○) and 20% (●) w/v. Results are the mean \pm SE of three replicates.

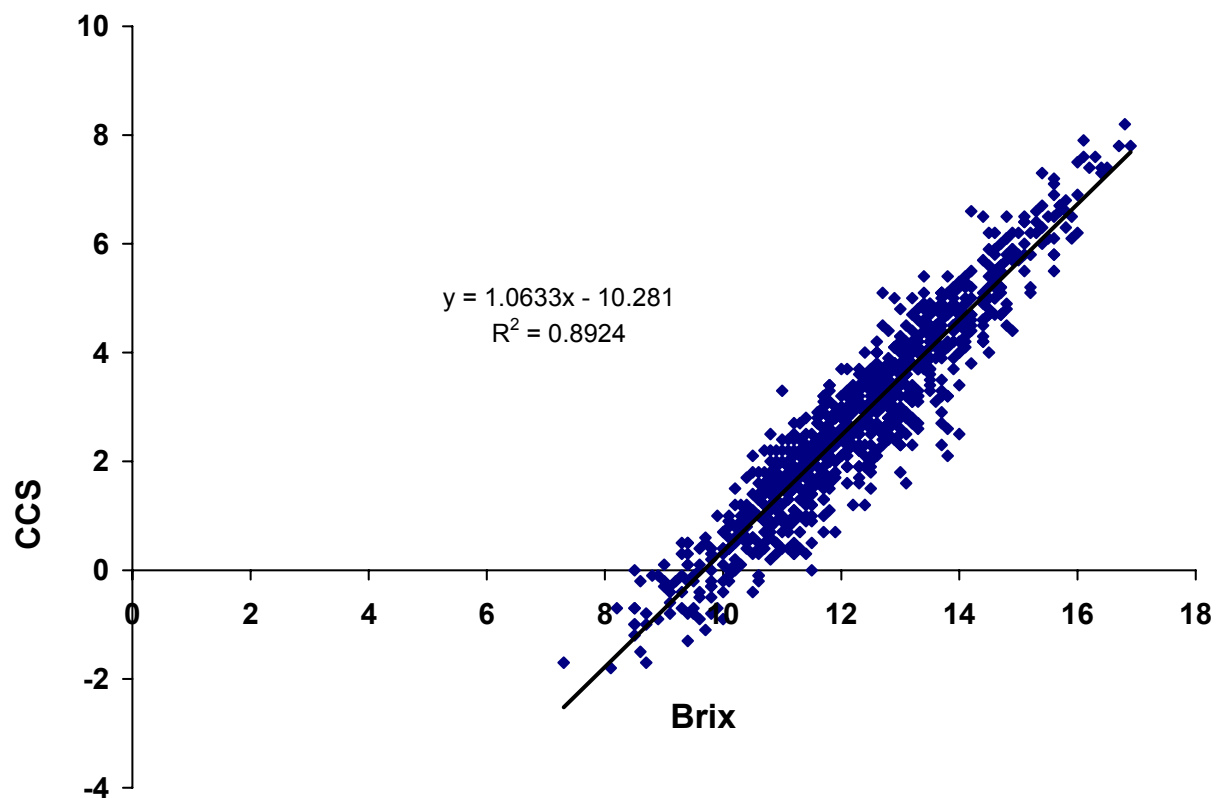


Figure 5. Early season Brix and CCS estimates from a sugarcane population. Juice was collected in March 2001, from 196 individuals with three or four plot replicates at 6 months of age.

Table 1. Estimated changes in CCS by altering the level of hexose and sucrose at various levels of Brix. Assumes 13 % fiber, glucose and fructose in equal amounts and Pol is derived from the calculations using standard sugar solutions (i.e. a 5.1 unit loss in Pol occurs with every 1 % sucrose converted to hexose (equal glucose and fructose)).

Hexose (%)	Sucrose	Brix (%)	Pol	CCS	Decrease in sucrose (%)	Decrease in CCS (%)
0	20	20	76.9	12.3	0.0	0.0
1	19	20	71.8	10.8	5.0	12.2
2	18	20	66.7	9.3	10.0	24.4
3	17	20	61.6	7.8	15.0	36.6
4	16	20	56.5	6.3	20.0	48.8
0	15	15	57.7	9.1	0.0	0.0
1	14	15	52.6	7.6	6.6	16.6
2	13	15	47.5	6.0	13.3	34.1
3	12	15	42.4	4.5	20.0	50.5
4	11	15	37.3	2.9	26.7	68.1
0	10	10	38.5	5.6	0.0	0.0
1	9	10	33.4	4.1	10.0	26.7
2	8	10	28.3	2.5	20.0	41.0
3	7	10	23.2	0.9	30.0	83.9
4	6	10	18.1	-0.6	40.0	110.7