SUGARCANE NUTRITIONAL ANALYSIS TO ENABLE FOOD SAFETY ASSESSMENT OF GM CULTIVARS—APPROACHES TO ESTABLISHING A BASELINE

By

AL RAE, GD BONNETT
CSIRO Plant Industry, St Lucia
anne.rae@csiro.au

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Abstract

GENETICALLY modified (GM) sugarcane cultivars are under development. Before these cultivars can be grown commercially and the products derived from them used, they will need approval by regulatory authorities. The pathway for regulation of the food derived from genetically modified sugarcane is discussed, in particular the likely requirements for the compositional analysis of sugarcane. Some of the components required for analysis are not normally measured. However, using data from some of the components of the plant that are frequently measured (sucrose, ash, nitrogen, fibre) it is possible to determine how factors such as genetics, season, geography, management (e.g. nutrition) and time of year may affect them. From this, a strategy for sampling crops and experiments to determine the range of values, particularly for the components not routinely measured such as fats and protein, can be defined. This strategy will facilitate sampling and measurement to document the range of compositions that could be expected from existing production systems. A set of baseline measurements that can be used for comparing the composition of GM plants to existing cultivars during the assessment process will be the end result.

Introduction

There has been increased activity around the world in the development of GM sugarcane (Bonnett *et al.,* 2010). As the potential to commercialise GM sugarcane gets closer, increased emphasis is being placed on the regulatory processes that need to be fulfilled in order to get approval to grow GM cultivars and export the sugar and other products derived from them. In Australia, the regulatory functions for GM crops are spread across several agencies, depending upon the modification (Mitchell, 2011). Approvals for GM crops are granted for a specific genetic 'event', defined as the insertion site of introduced DNA into the genome. The insertion site of the introduced DNA will be in different positions in the genome in each individual cell line that is regenerated into a GM plant. As most of the sugar from the Australian sugarcane crop is exported, approvals for growing GM sugarcane and approval for the sugar manufactured from it to be used as food will be required in export markets as well as in Australia. For example, GM sugar beet has been approved for commercial release in the USA, Canada and Japan but, in addition, at least one event has also been approved for use as food in 11 administrative territories (Table 1, James, 2009).

The potential in the near future for the commercialisation of GM sugarcane has also stimulated regulatory agencies around the world to prepare for the evaluation of GM sugarcane. This is apparent from the formation of Organisation for Economic Co-operation and Development (OECD) working groups to prepare both 'Consensus Document on the Biology of Sugarcane' and 'Consensus Document on Compositional Considerations for New Varieties of Sugarcane: Key Nutritional and Feed Nutrients and Anti-nutrients' documents. These documents will support the assessment of environmental impacts and the safety of food/feed respectively. Australia is leading

the development of these documents through the Office of the Gene Technology Regulator (OGTR) and Food Standards Australia and New Zealand (FSANZ), respectively.

Table 1—Countries where GM sugar beet has been approved for growing and/or use as food/feed (Data extracted from James, 2009).

Jurisdiction	Approval for growing		Approval for food/feed	
	Number of events	Year first event approved	Number of events	Year first event approved
Australia			2	2002
Canada	2	2001	2	2000
EU*			1	2007
Japan	1	2007	3	2001
Mexico			1	2006
New Zealand			2	2002
Philippines			2	2004
Russian Federation			2	2001
Singapore			1	2007
South Korea			1	2006
USA	3	1998	3	1998

* comprising 27 member states

Recent investigations in Australia have resulted in increased understanding of the sexual reproductive biology of sugarcane which will support the environmental assessment of GM sugarcane (Bonnett *et al.*, 2007, 2010). While some activity in this area remains to be completed, we have started the research necessary to create baseline information to support the assessment of food uses of GM sugarcane. This paper describes the approach taken.

General concepts of food safety assessment

Food safety is defined as 'providing assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use' (FAO/WHO, 1996). The assessment of food derived from biotechnology considers both intended changes caused by the modification and any unintended consequences, if they are present. For example, a GM plant variety that includes a gene conferring herbicide tolerance would be expected to produce the enzyme active in herbicide detoxification but would not be expected to show changes in any other traits. The accepted approach to satisfying this requirement is to demonstrate 'substantial equivalence' of the modified variety to a similar but unmodified variety with a history of safe use for food production (OECD, 1993; FAO/WHO, 2000).

The concept of 'substantial equivalence' implies that, if a new food is found to be substantially equivalent to an existing conventional food, it can be concluded to be as safe as the conventional food. If unintended changes have occurred, it would then be necessary to demonstrate that no harmful consequences would result from the modification.

The primary case that there are no unintended consequences is constructed by a weight of evidence approach using three 'pillars of regulation': (i) molecular characterisation of the genetic modification, (ii) phenotypic and morphological characterisation, (iii) compositional analysis.

Molecular characterisation

The intent of the molecular characterisation is to demonstrate that changes to the genetic code are limited only to the intended changes. The analysis required often includes the number and position of the changed elements, and the sequence context of the insertions. From this, the potential for creating new coding sequences that lead to the production of toxic or allergenic proteins can be assessed.

Phenotypic and morphological characterisation

Plant growth parameters can be used as indicators of developmental and physiological processes. The analysis would normally be performed on samples of the GM variety grown in the field together with conventional varieties as comparators. The measurements required comprise many of those that are normally made as agronomic performance is being assessed.

Compositional analysis

The nutritional components of the food derived from a GM cultivar should be substantially equivalent to its conventional counterpart, focussing on key nutrients, anti-nutrients and/or toxicants. To assist this comparison and to ensure consistency between countries, the OECD's Task Force for the Safety of Novel Foods and Feeds produces consensus documents on compositional characteristics of crops used as food and/or feed. The consensus documents include information on the use of plants for food and feed and the composition of food/feed products. The compositional analyses include macronutrients and micronutrients, as well as toxins and allergens specific to that crop species. Data ranges are normally included and these reflect the natural variability of the constituents in different plant varieties and in a range of geographical and climatic zones. Data need to be produced by reliable methods and ideally published in an international peer-reviewed journal. Consensus documents have been published for 17 species and more are in preparation including one for sugarcane. Those published are available at http://www.oecd.org/document/9/0,3343,en_2649_34391_1812041_1_1_1_1,00.html.

An example is provided by the document for maize (OECD, 2002a). In this document, the typical concentrations of a broad range of compounds were considered, together with the relevance of these compounds to the products of maize consumed by humans and animals. The primary nutritional components of a food substance, referred to as 'proximate analysis' include protein, fat, carbohydrates, total dietary fibre and ash. In addition, the document considered other nutrients:

- minerals (Na, K, Ca, P, Mg, Fe, Cu, Se and Zn)
- vitamins (Vit. A, Vit. B1 (thiamin), Vit. B2 (riboflavin), Vit. B6 (pyridoxine), Vit. C (ascorbic acid), Vit. E, folate and niacin)
- individual amino acids
- fatty acids (palmitic, stearic, oleic, linoleic and linolenic).

The anti-nutrients considered were phytic acid (myo-inositol hexa-phosphate), which reduces the bioavailability of phosphorus, DIMBOA (2,4-Dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one) which is a natural pesticide and raffinose, a non-digestible oligosaccharide which can cause abdominal discomfort.

Although they are neither nutrients nor anti-nutrients, the secondary metabolites, furfural, ferulic acid and *p*-coumaric acid were also considered because they are characteristic metabolites in maize and may act as useful comparators for determining equivalence.

The consensus document makes recommendations on the components to be analysed for assessment of a food product that originates from a GM maize cultivar. For assessment of whole kernel, comparison of proximates, minerals, vitamins, amino acids, fatty acids, phytic acid, raffinose, furfural, ferulic acid and *p*-coumaric acid is recommended. For assessment of specific products derived from maize kernels, the document recommends analysis of a limited range of components. For example, analysis of fatty acids is considered to be the key factor for assessment of maize oil, while analysis of proximates is suggested as appropriate for assessment of maize starch.

Similar analyses are suggested for crops such as rice, barley, sorghum and wheat, where whole grain is consumed. For crops such as sugar beet, where the products are refined sugar or molasses, and whole plant tissue is less likely to be consumed by humans, the OECD document recognises that the products are unlikely to contain high levels of modified proteins or DNA. For sugar beet, the document recommends that a modified variety be considered substantially equivalent if the composition of the root falls within the published range of concentrations of the key nutrients: ash (5.0–8.1% of dry matter (DM)), crude protein 4.7–6.8% DM), crude fibre (4.9–6.3% DM), sucrose (64.7–70.0% DM) and phosphorus (1.4–2.2% DM) (OECD, 2002b).

Regulation by Food Standards Australia and New Zealand (FSANZ)

In Australia, food safety regulation by FSANZ aligns with the OECD recommendations using substantial equivalence as a guide. However, the regulatory system has specific requirements for consideration of highly refined products from GM plants, such as oil or sugar. For these products, the assessment focuses on the part of the plant that the food is derived from. In the case of sugarcane, FSANZ considers that analysis of the refined sugar is not sufficient for two reasons:

1) The composition of refined sugar is close to 100% sucrose and any variation in the impurities is more likely to reflect differences in the refining process rather than differences derived from the modified plant tissue.

2) When food safety approval is granted, the approval covers all products and potential products from the modified plant. Although unprocessed sugarcane is not widespread as a food source in Australia, there are niche markets for juice, and juice and pulp are routinely consumed in other countries. Therefore, food safety assessment needs to consider the potential use of other parts of the sugarcane plant.

For these reasons, the assessment is likely to be performed on the sugarcane stalk, as this is more likely to reflect the composition of the whole plant. In a food safety assessment, FSANZ uses information on the composition of conventional varieties from a variety of sources including the OECD consensus document, data submitted by the proponents and published information. The key constituents that are likely to be included in the assessment of sugarcane are shown in Table 2.

Table 2—Components, food and feed that are likely to be required for compositional analysis of a GM sugarcane stalk.

Analysis	Components
Food	Moisture, crude fibre (% insoluble dry matter), crude protein, fat (as ether extract), ash, sucrose, total sugars*
Feed[+]	Neutral detergent fibre (NDF) and acid detergent fibre (ADF)

[*]free monosaccharides and disaccharides. For sugarcane, these primarily consist of sucrose plus lesser amounts of reducing sugars (glucose and fructose). [+] Additional measurements for feed.

How should a baseline be defined for substantial equivalence?

A suitable reference is required for substantial equivalence comparisons. If the baseline constructed is too narrow, assessed samples may frequently fall out of the range, for reasons not associated with the genetic change, consequently raising potential safety concerns that would require further investigation. Baker *et al.* (2006) compared the amino acid composition of wheat that had been transformed to alter seed protein composition and noted that, while there were differences found through direct comparison to the untransformed lines in some sites, it was less

than the range of the controls across the sites tested. Similarly, Moller and Bak (2005) have highlighted that any comparisons made using substantial equivalence need to place the results in the context of the other sources of variation affecting the components under study. Consequently, we propose to construct a range for the major compositional components that would capture the major sources of variation likely to be encountered.

It is impractical to construct the baseline by analysing the composition of all commercial sugarcane varieties under all possible growing conditions. Therefore we propose to select samples that will give the best possible estimate of the normal ranges encountered in the Australian environment. To guide the selection of these samples, we have examined the existing records of sugarcane composition to determine the factors that have most influence on the composition, such as plant variety, time of harvest, growing region and management practices.

Some of the proposed components (e.g. crude protein and fat) are rarely measured in experiments designed to evaluate the performance of potential cultivars, or the effects of agronomic treatments on yield and sugar content. Fortunately, many of the other components are routinely measured. Analysis of the likely ranges of these components and the sources of variation that give rise to the largest differences can be used to inform a sampling strategy that encompasses the range of these constituents likely to be found across a range of sugarcane growing environments and practices.

Sources of variation

The three major components of sugarcane stalks are moisture, sucrose and fibre. The remaining components are often referred to in an industry context as impurities and they mostly remain in molasses along with the sucrose that cannot be extracted.

There are many factors that affect the sucrose content of sugarcane stalks. CCS, the industry estimate of sucrose, can vary with season (e.g. the seasonal average for Mulgrave from 1903–1996 varied from about 11.8 – 15.7; Pope, 1997); mill region (the 1994 seasonal average of all individual mills across Queensland was 11.99–15.77; BSES, 1995); and time of the year (8–17 measured on whole stalks from May to September; Cox *et al.,* 1998). Measurements of CCS made at the mill are not made on just stalk material and so underestimate stalk CCS. While CCS will also vary with many other factors, these are all probably captured within the ranges quoted above. The data from Cox *et al*. (1998) include samples taken outside the normal harvested season in May. However, the range of 8–17 probably encompasses the range seen at most Australian sugar mills. Cane samples with CCS values below 7 would not be accepted by mills because they indicate such low sucrose content that it becomes uneconomical to process the cane and values above 17, though desirable, would be rarely seen (Inman-Bamber *et al.,* 2011).

Increased sucrose content is normally at the expense of reducing sugars and moisture (Bonnett *et al.*, 2006). Moisture in sugarcane stalks will also be highly variable. Berding and Marston (2010) showed in measurements of whole stalks that moisture content from 40 trials in 2009 in the northern region, varied between 61 and 72%. In the same samples, fibre varied between 9 and 18%. In a range of cultivars across the Mackay district sampled in September 1988, fibre ranged from 10.5 to 13.9% (Ivin and Doyle, 1989), well within the range generated by Berding and Marston (2010).

Therefore, sampling stalks early and later in the season is likely to cover the range of sugar contents, moisture and fibre contents found in cane presented to mills. However, the real value of this sampling strategy will be an attempt to cover the range of other, less well studied components. Below, we discuss what is known about the variation and its causes in these lesser-studied components.

***Protein*:** The protein content of food products is frequently estimated from the total nitrogen content which is measured by the Kjeldahl assay or a similar method. The average nitrogen content of proteins is assumed to be approximately 16%, resulting in a conversion factor of [N] × 6.25 (FAO, 2003). Some indication of the likely variation in protein content of the sugarcane stalk can be derived from studies of nitrogen content.

Keating *et al.* (1999) studied variation in the content and chemical form of nitrogen in cane samples that were supplied with different amounts of N fertiliser from eight trial sites between Grafton and Ingham. The aim of the work was to design production systems that optimise profitability by minimising input costs and by changing the composition of the sugarcane stalk towards more profitable components. The total N content of whole cane stalks for the trials sampled was 0.1–0.6% on a dry matter basis (Table 3), equivalent to 0.63–3.75% protein using the above conversion factor.

The study used the amino acid N content of juice (amino N) to dissect the influence of variables such as fertiliser treatment, crop age and water stress. Although the proportion of total stalk N found in juice can be as low as 30%, in most cases 50–70% of the total stalk N was recovered in the juice, suggesting that juice N was a reasonable indicator of stalk N content. The amino N ranged from 100–900 μg N/mL juice (Table 3), with the higher contents found in trials receiving high fertiliser inputs or trials where cane yield was limited by environmental factors such as water stress. The crop cycle was a significant influence on amino N in juice, as cane from plant crops had amino N levels that were 40–50% higher than cane from ratoon crops. Within the range of N contents found, no clear influence of variety was found although some regional effects were apparent. Overall, the results suggest that environmental and management factors will be the greatest source of variation in protein content of the sugarcane stalk.

***Ash*:** Nitrogen also affects the level of ash, measured as conductivity of juice. For example, the addition of 280 kg N/ ha reduced the conductivity/impurity fraction by 20% compared to a control treatment (Jackson *et al.,* 2008). In the same study, conductivity/impurity was increased by about 30% when fertiliser potassium was increased from 0 to 260 kg K/ha (Table 3).

Table 3—Some effects of different sources of variation on the ash and nitrogen content of sugarcane stalks and juice.

Source of variation	Component	
	Nitrogen (as total N or amino N)	Ash
Nitrogen fertiliser	100–900 □g amino N/mL juice[1]	Decrease by 20%[2]
Potassium fertiliser		Increase of 30%[2]
Region/Variety	0.1–0.6% total N in dry stalk[1]	0.55–0.72% of first expressed juice[3]
Crop cycle	40–50% higher amino N in juice in plant crop	

[1]Keating *et al.* (1999); [2]Jackson *et al.* (2008); [3] Kingston and Kirby (1979);

Different geographic regions of the Australian sugarcane industry are spread along a large range of latitudes, consequently experiencing considerable variation in rainfall, temperature and other climatic factors. In addition, soil types vary both within and between regions. A good example of how this affects sugarcane composition is demonstrated by the levels of ash. This can vary within and between regions based on several factors such as water quality. Kingston and Kirby (1979) found that in cane delivered to the Qunaba mill east of Bundaberg, ash levels in first expressed juice varied between 0.55 and 0.72% when samples were grouped on the basis of water quality used for

irrigation (Table 3). This variation would be expected to be reflected in the ash content of whole stems.

***Fat*:** There are very few measures of fat (ether extract) of sugarcane stalks. One study reported 0.81% fat in dry matter of mature cane (16 months old) and 1.10% in immature cane (Banda and Valdez, 1976). The paucity of studies means that there are no clues as to how environmental or management conditions may affect the fat composition of sugarcane.

***Total sugars*:** The measurement of total sugars is highly correlated with Brix, which is often measured. The study of Berding and Marston (2010) showed a range of Brix between 18 and 27%. It is anticipated that sampling to encompass the range of sucrose content will also mean we will capture a large range of total sugars.

***Neutral detergent fibre (NDF) and acid detergent fibre (ADF)*:** In some countries, such as Brazil, sugarcane is an important source of feed for ruminant animals, especially during the dry season. When biomass for feed is analysed by sequential chemical extraction, the fraction remaining after neutral detergent extraction (NDF) comprises most of the fibre (lignin, hemicellulose and cellulose but not pectin), while further extraction with acid detergent results in a fraction (ADF) comprising only the cellulose and lignin components of the fibre. NDF is used as a measure for bulk forage volume, while ADF is related to the energy value of the forage, since digestibility decreases as ADF increases. The nutritional value of different sugarcane varieties as feed has been compared and several studies have been published in Brazilian scientific journals. Although it is not always possible to isolate stalk composition from these studies, they can give some indication on the likely sources of variation in composition in whole plants. In a study by de Souza Franca *et al.* (2005), ten varieties that were grown on the same site showed significant variation in content of crude protein (although the total amount was low) and in NDF content. Similar studies by Azevedo *et al.* (2003a) and Rodrigues (1997) found significant differences in NDF. The influence of environment and management practices on nutritional composition has been less studied. Azevedo *et al.* (2003b) and Fernandes *et al.* (2003) showed that the time of harvest of sugarcane affected the content of crude protein and NDF. Although we were not able to find similar work in sugarcane, the effect of irrigation on the nutritional value of two forage grasses, *Panicum maximum* and *Pennisetum purpureum* has been analysed (Palieraqui *et al.,* 2006). The results showed that irrigation did not affect the content of crude protein, ash, NDF or ADF. The lack of information available for sugarcane suggests that a study to investigate the effects of environment on nutritional content would be worthwhile.

Strategy for sampling to capture sources of variation

Some interesting insights into the major sources of variation in composition have been gained by examining the records of frequently analysed components. Although genetic background would seem, intuitively, to be a major factor, the results show that environmental conditions and management practices are actually more important. For the commonly analysed components of CCS, water and fibre, the time of harvest had the greatest influence. Although fewer records were available for the smaller components, protein and ash, the major influences appeared to be the quality of soil and water, including fertiliser applications.

In the light of these results, samples from a small number of genotypes grown across a wide variety of regions will be collected. Sampling at the start and middle of the harvesting season will probably capture a large range of compositions for total sugars, sucrose, moisture and fibre. However, for some components such as protein and ash, a more targeted sampling of crops that have had high levels of N applied and treatments that increase ash content may be required to capture the range. When preliminary analyses are complete, these can be used to see what further

sampling, if any, would be required to extend the analyses of fat, NDF and ADF values to capture the likely range in sugarcane stalks grown across the Australian industry.

Conclusions

A significant component of the assessment of the food safety of proposed GM sugarcane releases will be the compositional analysis of sugarcane stalks. The number of components to be analysed is likely to be much less than for grains and other less refined food products. It is important that a baseline for comparison encompasses the likely range of compositions encountered in Australian growing conditions. Examining records of sugarcane composition suggests that environmentally induced variations are generally greater than variation among commercial cultivars. A two-stage strategy for sampling is proposed to capture this variation: (i) initially sampling a small number of varieties from different regions at the start and middle of the harvest season, and (ii) targeting sampling of crops in particular environments to supplement the range if necessary. The outcome of this work should be a set of data that will be useful in testing the substantial equivalence of GM and conventional varieties.

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REFERENCES

Azevedo JAG, Pereira PCS, Carneiro AC, Quieroz MHP, Barbosa AM, Fernandes AM, Renno FP (2003) Avaliação da divergência nutricional de variedades de cana-de-açúcar (*Saccharum* spp.). *Revista Brasileir de Zootecnia* **32**, 1431–1442.

Azevedo JAG, Pereira PCS, Carneiro AC, Quieroz MHP, Barbosa AM, Fernandes AM, Renno FP (2003b) Composição químico-bromatológica, fracionamento de carboidratos e cinética da degradação in vitro da fibra de três variedades de cana-de-açúcar (*Saccharum* spp.). *Revista Brasileir de Zootecnia* **32**, 1443–1453.

Baker JM, Hawkins ND, Ward JL, Lovegrove A, Napier JA, Shewry PR, Beale MH (2006) A metabolomic study of substantial equivalence of field-grown genetically modified wheat. *Plant Biotechnology Journal* **4**, 381–392.

Banda M, Valdez RE (1976) Effect of stage of maturity on nutritive value of sugar cane. *Tropical Animal Production* **1**, 94–97.

Berding N, Marston DH (2010) Operational validation of the efficiency of spectracane™, a high-speed analytical system for sugarcane quality components. *Proceedings of the Australian Society of Sugar Cane Technologists* **32**, 445–459.

Bonnett GD, Hewitt ML, Glassop D (2006) The effects of high temperature on the growth and composition of sugarcane internodes. *Australian Journal of Agricultural Research*. **57,** 1087–1095.

Bonnett GD, Berding N, Morgan T, Fitzgerald P (2007) Implementation of genetically modified sugarcane – the need for a better understanding of sexual reproduction. *Proceedings of the Australian Society of Sugar Cane Technologists* **29**, 258–266.

Bonnett GD, Olivares-Villegas JJ, Berding N, Morgan T (2010) Sugarcane sexual reproduction in a commercial environment: Research to underpin regulatory decisions for Genetically Modified sugarcane. *Proceedings of the Australian Society of Sugar Cane Technologists* **32**, 1–9.

BSES (1995) BSES 95th Annual report. BSES, Queensland.

Cox MC, Ridge DR, Hussey B (1998) Optimum time of harvest for high early-CCS sugar varieties. *Proceedings of the Australian Society of Sugar Cane Technologists* **20**, 218–223.

de Souza França AF, S Queiroz Santos Mello, B Rosa, A de los Reyes Borjas, S Piereira Mundim, MR Ferreira Maghalães, TR Aparecido de Matos, J Gonçalves dos Reis (2005) Avaliação

do Potencial Produtivo e das Características Químico-Bromatológicas de Nove Variedades de Cana-de-Açúcar Irrigada. *Livestock Research for Rural Development* **17**, Art #7

FAO (2003) Food energy – methods of analysis and conversion factors. Food and Nutrition Paper 77, Food and Agriculture Organisation of the United Nations, Rome. ftp://ftp.fao.org/docrep/fao/006/y5022e/y5022e00.pdf

FAO/WHO (1996) Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety. ftp://ftp.fao.org/es/esn/food/biotechnology.pdf

FAO/WHO (2000). Safety aspects of genetically modified foods of plant origin. Report of a joint FAO/WHO Expert consultation on foods derived from Biotechnology. Geneva, Rome, FAO.

Fernandes AM de Queiroz AC, Pereira JC, Lana RD, Barbosa MHP, de Fonseca DM, Detmann E, Cabral LD, Pereira ES, Vittori A (2003) Composição químico-bromatológica de variedades de cana-de-açúcar (*Saccharum* spp L.) com diferentes ciclos de produção (precoce e intermediário) em três idades de corte. *Revista Brasileir de Zootecnia* **32**, 977–985.

Ivin PC, Doyle CD (1989) Some measurements of the effect of tops and trash on cane quality. *Proceedings of the Australian Society of Sugar Cane Technologists* **11**, 1–7.

Inman-Bamber G, Bonnett G, Jackson P, Morgan T (2011) Have we reached peak CCS? *Proceedings of the Australian Society of Sugar Cane Technologists* **33**, This Proceedings.

Jackson PA, Schroeder BL, Rattey AR, Wood A, O'Shea MG (2008) Management of Ash/impurity ratio in sugarcane: relative effects of genotypes and N and K fertiliser rates. *Australian Journal of Agricultural Research* **59**, 795–801.

James C (2009) Global status of commercialised Biotech/GM crops:2009. ISSA Brief No. 41. ISAAA: Ithaca, NY.

Keating BA, Kingston G, Wood AW, Berding N, Muchow RC (1999) Monitoring nitrogen at the mill to guide N fertilisation practice on farm. *Proceedings of the Australian Society of Sugar Cane Technologists* **21**, 10–19.

Kingston G, Kirby LK (1979) Ash in juice – A supply area survey. *Proceedings of the Australian Society of Sugar Cane Technologists* **1**, 61–69.

Mitchell H (2011) GM^4 Regulation of genetically modified (GM) sugarcane in Australia. *Proceedings of the Australian Society of Sugar Cane Technologists* **33**, This Proceedings.

Moller BL, Bak S (2005) Response to Kutchan: Genetically engineering natural variation and substantial equivalence. *Trends in Biotechnology* **23**, 383–384.

OECD (1993) Safety evaluation of foods derived through modern Biotechnology: Concepts and principles. OECD, Paris,.

OECD (2002a) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. http://www.oecd.org/document/9/0,3343,en_2649_34391_1812041_1_1_1_1,00.html

OECD (2002b) Consensus document on compositional considerations for new varieties of sugar beet: key food and feed nutrients and antinutrients. http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono(2002)4&doclanguage=en

Palieraqui JGB,Fontes CAA, Ribeiro EG, Coser AC, Martins CE, Fernandes AM (2006) Influência da irrigação sobre a disponibilidade, a composição química, a digestibilidade e o consumo dos capins mombaça e napier. *Revista Brasileir de Zootecnia* **35**, 2381–2387.

Pope GM (1997) Mulgrave CCS declines to record lows in 1993 and 1995. *Proceedings of the Australian Society of Sugar Cane Technologists* **19**, 30–37.

Rodrigues A de A, Primavesi O, Esteves SN (1997) Efeito da qualidade de variedades de cana-de-açúcar sobre seu valor como alimento para bovinos. *Pesquisa Agropecuária Brasileira* **32**, 1333–1338.