Sugar Research and Development Corporation

PROJECT CTA045

IMPROVING CCS IN THE WET TROPICS VIA BLOCK-SPECIFIC MONITORING OF N IN CANE DELIVERED TO THE MILL

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Project Title: Improving CCS in the wet tropics via block-specific monitoring of N in cane delivered to the mill

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Statement of Confidentiality:

No part of this report is considered confidential.

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1. Executive summary

Nitrogen fertilisers cost the Australian sugarcane industry approximately $80M p.a. While nitrogen applications are essential for profitable and sustainable intensive cropping, losses of nitrogen from the production system degrade soils through acidification and pose a significant environmental risk to surface freshwaters, groundwaters and marine systems. Efficient nitrogen management in sugarcane systems has been hampered by the lack of a relevant, cost-effective method of assessing the nitrogen status of sugarcane crops and/or soils. Other projects have demonstrated how the nitrogen status of cane delivered to mills can be an effective indicator of the nitrogen supply under which this cane was produced (the "N at the Mill" concept). The amino-N component of the total-N in the cane has been the primary target for this monitoring, as earlier studies have demonstrated this component shows higher levels of sensitivity to external N supply than does total-N. Such information can be potentially used to adjust nitrogen management in future crops on a block-specific basis. Other studies have demonstrated this approach is most effective at detecting situations of excess nitrogen supply. It is under such situations that a diagnostic test is most needed, as they are the conditions that pose greatest environmental risk and where traditional tests (such as leaf tissue analysis) are least effective.

This project has focused almost entirely on overcoming the challenge of achieving a cost-effective method of monitoring nitrogen in cane as it is processed in the mill. The target methodology has been Near-Infrared Spectrometry (NIR), and the challenge has been to develop a calibration methodology that will provide sufficiently robust estimates of amino-N levels in cane supply for the intended application. The initial objectives were broader than this and included a major focus on working with farmers in action learning settings to demonstrate the value of this additional information in farm management. These broader objectives were scaled back during the life of the project as technical obstacles to achieving sufficient accuracy in the on-line monitoring of amino-N were encountered.

Specific achievements with respect to the goal of a cost-effective on-line monitoring technique include;

The majority of project outputs relate to key understandings relevant to the objective of the development of mill-scale calibration of NIR as a practical tool in the routine monitoring of amino-N levels in cane supply. These outputs include;

- Transmission NIR was shown to be capable of measuring amino-N levels in first expressed juice, but only after the juice was filtered to remove the high levels of colloidal material generally present. On line filtering of first expressed juice was deemed problematical and inconsistent with industry moves in the direction of reflectance NIR of whole cane supply (i.e., Cane Analysis System NIR). Hence the project placed its focus on reflectance NIR approaches on whole cane supply.
- Three separate attempts confirmed our inability to calibrate NIR spectra recorded from whole cane supply, with amino-N levels measured in the laboratory on first expressed juice. The reasons for these calibration failures were investigated, in terms of robustness of the laboratory procedures in amino-N measurement and nature of the within mill processes that might influence the overall calibration precision.

- In terms of robustness of laboratory procedures, the project has shown;
    - Various pre-filtering techniques were not found to significantly influence the amino-N determination.
    - The ninhydrin assay was shown to slightly underestimate the amino-N concentration compared to an assay based on full determination and summation of individual amino-acids by HPLC methods. However, the correlations remain tight and the ninhydrin assay method was not considered to be a significant factor introducing variability into the Cane Analysis System (CAS) NIR calibration process.
    - An improved conversion equation was deemed necessary for relating ninhydrin determined amino-N to total amino N. A new conversion equation was proposed, but needs additional samples collected from 2002 analysed via HPLC to confirm its robustness. This equation is consistent with our basic understanding that indicates the amino-N in sugarcane stems is dominated by the amino-acid asparagine, which contains 2 atoms of nitrogen in every amino-acid molecule.
- In terms of nature of the within-mill processes that might influence the NIR calibration process, the project has shown;
    - The amino-N concentrations in first expressed juice is not fully representative of the total amino-N in the whole cane supply.
    - This “non-representativeness” relates more to the cane preparation technique (i.e. the level of fibration of the cane stem tissues and cells) than to the pressure applied in the of juice extraction process. Much better progress in NIR calibration development was possible when a combination of the Jeffco Cutter Grinder was used with a Carver Press to extract amino-N in a juice sample than proved possible with first expressed juice.
    - Methanol extracts of total amino-N in whole cane samples prepared by Jeffco Cutter Grinder confirmed that calibration was possible when a consistent extraction methodology was deployed.
- Significant enhancements in the calibration of CAS NIR in estimating amino-N levels in cane supply were achieved via log transformations of the laboratory determined data to account for the non-normal distribution in the amino-N concentrations.
- As an alternative to amino-N determination by CAS NIR, an alternative calibration of total-N was demonstrated. This could prove useful in future work that aims to use total N removal in harvested product as an explicit source of information in guiding N fertiliser inputs.
- Investigations of the effects of addition of extraneous matter to the determination of amino-N levels in cane stems revealed little effect on amino-N concentrations, but larger reductions in the quantities of juice that could be extracted. These physical effects of extraneous matter on juice extraction could have added to the difficulties encountered in our early attempts to calibrate NIR using first expressed juice. This provides further rationale to develop NIR calibration based on a direct measure of total amino-N in the whole cane sample, and not introduce the additional variable of the juice extraction process in the No 1 mill.

Specific achievement with respect to the broader goals of deploying this "N at the Mill" monitoring technique in a number of farm-mill settings include;

- Project objectives that related to investigating the relationships between amino-N levels in cane stems and CCS levels were constrained by the methodological challenges encountered in the development of robust NIR calibrations. Nevertheless, limited investigations based on direct amino-N laboratory measurements have shown situations in which high amino-N levels have been associated with low CCS. Many factors influence CCS and cause and effect is difficult to ascertain. However, with the more extensive data capture possible with NIR based assessment, we would expect improved insights to emerge.

Additional outputs relate to strategies for communicating amino-N information to growers. These include;

- Through a participatory process with growers in Mossman and Mulgrave mill areas, two reporting mechanisms were developed and captured in a communications plan. This plan has the following features;
    - Within season reporting of amino-N via regular rake reports.
    - End of season "whole farm" performance of amino-N monitoring via block specific maps.
    - A framework of reporting based upon four categories (low, target-low, target-high and excess), with specific guidance to growers associated with each category.
    - A document titled "Guidelines for Cane Farmers on use of Amino Nitrogen Data" produced in consultation with growers and productivity staff from Mossman and Mulgrave mill areas to assist in interpretation of "N at the Mill" monitoring.

In summary, the technical obstacles to developing a useful calibration method for detecting levels of amino-N in cane supply have been overcome. The latest validation exhibits coefficients of correlation (r^2) in the order of 0.8. These validations are based on 252 samples from Mossman, Mulgrave and Tully mills over the 2001 and 2002 seasons, however these samples are not fully independent of the samples used to build the initial calibration, deriving from the same dataset, but being redundant data not used in the calibration. Given that the intention is to base actions on broad ranges of amino-N levels (i.e., excess, upper-target, lower-target, deficient), predictive accuracy at this level is considered sufficient for wider application of this monitoring approach. Experience with other NIR applications suggests that this calibration will improve as additional data become available. A mechanism needs to be established where ongoing sample collection and analysis for amino-N occurs at each mill with NIR to validate and further improve the amino-N calibration.

We suggest the monitoring technique is now ready for wider application, initially in the mill areas featuring in the calibration development. We believe this is a timely development, as the sugar industry is currently looking for opportunities to demonstrate it takes its responsibilities in environmental protection seriously. Through deployment of the "N at the Mill" approach, the industry can demonstrate that the N status of every block of cane in a mill area is being

assessed annually and this information is being used to fine-tune N fertiliser inputs. In this way, the industry will be able to build up the evidence to support its environmental management credentials. We suggest the “N at the Mill” technique could be deployed in a case study mode in the Mossman, Mulgrave, and Tully mill districts as early as the upcoming 2003 crushing season or in the 2004 season if additional time is needed to put implementation arrangements in place.

2. Background

The Australian sugar industry benefits from the application of nitrogen fertiliser to sugarcane. Sugarcane produces large quantities of biomass, often in excess of 100 tonnes per hectare, for which large amounts of nitrogen fertiliser application are necessary. During 2000 approximately 90 000 tonnes of nitrogen, worth approximately $80 million, was applied to the Australian crop (Calcino 2001, Chundleigh and Simpson 2001). Evidence exists that nitrogen application rates to sugarcane are in excess of what is required (Keating *et al.* 1993, Catchpoole and Keating 1995). Thorburn *et al.* (2003) illustrates how from 1965 to the present, N fertiliser application rates have increased from approximately 120 kg/ha to close to 200 kg/ha. Congruently the N input ratio has also increased from approximately 1.4 to 2.5 kg of applied N/tonne of cane. Keating *et al.* (1997) suggest an N input ratio of 1.4 is adequate to fertilise a sugarcane crop.

Over application of nitrogen fertiliser can lead to increased losses of nitrogen to the environment. These losses can impact negatively on the environment through increased nitrogen loads in ground water (Weier *et al.* 1996a), surface water (Bramley and Roth 2002) and the atmosphere (Weier *et al.* 1996b). Over application of nitrogen fertiliser costs growers time and money to apply (Wood *et al.* 1997).

The commercial cane sugar (CCS) content of sugarcane in the wet tropics has exhibited a well documented decreasing trend between 1960 and 1995 (Leslie and Wilson 1996). This decreasing trend is identified from “all of mill’ averages, therefore the decreases reported (10-15%) may be greater when applied to specific locations and/or situations. A major contributor to this decline was found to be a decrease in the sugar percentage of the total solids yield from sugarcane (sugar as a percentage of sugarcane dry weight). Decreasing sugar percentage can be attributed to a number of factors including an increase in extraneous matter in cane supply, which has the effect of diluting the sugar content of cane juice. There is evidence increased nitrogen supply to sugarcane has the effect of decreasing the CCS of cane (Muchow *et al.* 1996). Low CCS associated with high nitrogen supply may be due to increased nitrogen causing a related increase in crop trash and sucker levels.

A reliable, inexpensive, diagnostic measure of plant nitrogen is not readily available to sugarcane growers, particularly one adapted to identifying instances of excess nitrogen. Soil and leaf analysis techniques exist (Schroeder *et al.* 1998, Schroeder *et al.* 1999), but Keating *et al.* (1999) argue they are costly, time consuming, not likely to be carried out universally and are generally more suited to identifying crop nutrient deficiencies (Schroeder *et al.* 1999).

Christiansen *et al.* (2001) concluded growers relied heavily on their own knowledge when making decisions on crop fertiliser usage. This could help interpret the situation that has been reached where average crop nitrogen applications are 25% greater than industry recommendations (Thorburn *et al.* 2003). The maxim of ‘if you can’t measure it, you can’t manage it’ applies to nitrogen in sugarcane.

Sugarcane has been shown to respond to high nitrogen levels by increasing the uptake of nitrogen into the plant (Muchow and Robertson 1994), with the bulk of nitrogen taken up superfluous to immediate crop requirements stored in

the stem. The SRDC supported pilot project CSC21s demonstrated nitrogen associated with amino acids (amino-N) was one of these storage mechanisms used by the plant. The outputs of project CSC21s were:

- Approximately 60% of the N contained within cane stalks is found in the pressed juice.
- The proportion of the juice N present as amino acids ranges from 20% to more than 90% depending on the overall N supply.
- The amino acid asparagine was the dominant form of amino-N in cane stems, making up in excess of 80% of the amino-N pool under conditions of excess N supply.
- Amino acid N in cane juice responds strongly to N supply, particularly N excess.

These findings provide the foundations for using a measure of amino-N as a diagnostic tool for the nitrogen status of a sugarcane crop. The SRDC supported project CTA029 extended the knowledge base of monitoring amino-N and established:

- That variation in N supply (such as arises from differential N fertilisation practice) has the dominant influence on the amino-N concentration in cane stems at harvest time.
- That all the varieties tested to date exhibit the phenomenon of elevated amino-N levels in cane stems/juice in response to excess N supply, although there are some varietal differences in the absolute concentrations that are observed under elevated N supply.
- That water stress has a modest influence, in the form of elevated amino-N concentrations in crops that have experienced yield-limiting levels of water deficit.
- That amino-N levels in cane stems exhibit a modest downward trend as a crop ages, but the age related differences within a typical harvest window are small and of little consequence in terms of interpretation of amino-N monitoring information.
- That all factors impinging on amino-N concentrations in cane stems can be interpreted via a conceptual model of demand for N for new growth (biomass) and supply of N from N compounds stored in stem tissues (principally amino-N) and where relevant, continued uptake from soil sources.
- A diagnostic relationship between relative sugar yield and amino-N concentrations which can be used to define three zones of N supply, namely excess, target and potentially deficient.

The next developmental step in the amino-N monitoring process is to identify and develop a rapid, reliable and universal tool for monitoring amino-N. Near Infrared Spectroscopy (NIR) technology provides this opportunity. Advantages of NIR for monitoring amino-N are:

- Sample analysis is universal, and non destructive
- No sample preparation is required
- Real time results are delivered
- Results are related to the mill rake recording system

Staunton *et al.* (1999) describe an NIR system installed in four wet tropics sugar mill that scans prepared cane prior to it entering number one mill. Attempts to calibrate amino-N using this NIR system are described in this report.

3. Objectives

This project seeks to implement a scheme for monitoring the nitrogen status of all cane crops in a wet tropics mill district and aims to work with growers in the use of this information to improve CCS and reduce the risks associated with the overuse of nitrogen fertiliser.

The project aims to build on the SRDC supported pilot project "CSC21s – Monitoring N at the Mill" and the project "CTA029 – Monitoring N at the Mill". These projects demonstrated the N composition of cane juice, particularly N associated with amino acids, reflects the N status of the farm block supplying the cane. These projects present the opportunity to use this monitoring technique to firstly address the low CCS issue (in particular the contribution of high N on CCS) and secondly the real time implementation of the monitoring technique at an entire mill scale in the wet tropics.

The specific objectives of this project, as stated in the original project document are:

- Work with growers to identify ways in which block-specific information on crop N status can be used as a benchmark tool to improve N management for optimal CCS in the wet tropics.
- Develop and validate mill scale calibration of NIR as a practical tool in routine monitoring of amino-N levels in cane supply.
- Identify relationships between crop N status (as determined by NIR based amino-N assays) and CCS at the block scale.
- Explore the impact of the different components of cane supply (sound cane, suckers and extraneous matter) on the amino-N monitoring technique.

These objectives depend on the assumption calibration of NIR for amino-N would be accomplished early in the projects life. As the project evolved it became increasingly apparent to the project team calibration of NIR for amino-N was not straightforward. These concerns have been reported to SRDC through the milestone reports 4 and 6 (Appendices 21 and 23) and a milestone variation request (Appendices 30 and 31). Commensurate with SRDC milestone requirements the project established a project consultative reference group made up of growers, millers and researchers early in the project life to steer the projects activities. A role statement for the project consultative group is provided in Appendix 3. Once it became apparent CAS NIR was challenging to calibrate for amino-N the project reference group made a decision that the project team should pursue a robust calibration for amino-N as a priority and limit the communication of amino-N data to growers, even though grower interest in the outcomes of the project were high (Appendix 32). The reasoning behind pursuing a calibration for amino-N as a priority is that the potential benefits to the industry of universal feedback of amino-N to growers cannot be achieved without an NIR calibration.

Through the life of this project the objectives have evolved from the original set of objectives stated previously to a new set where the development of a robust calibration of NIR for amino-N became the priority. The project team has kept SRDC informed via the milestone reports of the difficulties in achieving a satisfactory calibration for amino-N using the CAS NIR and the decision of the project team to prioritise the quest for a robust calibration (Appendices 19, 20, 21 and 22). Milestones have been renegotiated with SRDC that reflect this situation (Appendices 30 and 31). The project has essentially operated under a modified set of objectives. The evolved set of objectives pursued by the project team, and reported in this report, in priority order, are:

1. Develop and validate mill scale calibration of NIR as a practical tool in routine monitoring of amino-N or total N levels in cane supply.
2. Develop a communication plan identifying the key steps necessary for successfully implementing a nitrogen monitoring technique to an entire mill region.
3. Identify relationships between crop N status (as determined by laboratory based amino-N assays) and CCS at the block scale.
4. Explore the impact of the different components of cane supply (sound cane, suckers and extraneous matter) on the amino-N monitoring technique.

The extent to which the objectives have been met has been influenced by the prioritisation of developing a robust calibration equation for NIR. The project team have been working on developing a calibration equation for the *Cane Analysis System (CAS)* NIR installed at four wet tropics sugar mills. Achieving a robust calibration for CAS NIR would allow communication of amino-N monitoring to growers supplying approximately five million tonnes of cane annually. Experimental NIR systems had robust calibration equations developed, however these do not allow for communication to growers, as they are not routinely used in the mill. The current calibration developed for CAS NIR has the project team 'cautiously optimistic' that CAS NIR is able to differentiate between low, target and excess amino-N ranges. This calibration needs to be validated using a dataset that was not used in the calibration development, such as one sampled from the 2003 season. Validation using a new dataset is essential before the project team can recommend communication of amino-N to growers from CAS NIR.

4. Methodology

4.1 Objective 1: Develop and validate mill scale calibration of NIR as a practical tool in routine monitoring of amino-N or total N levels in cane supply

Attempts have been made to calibrate NIR for amino-N, asparagine and total N. Amino-N calibrations are attempted on spectra taken on both sugarcane juice and cane fibre. Asparagine calibrations are attempted on sugarcane juice spectra. Total N calibrations are attempted on spectra taken on cane fibre.

4.1.1 Amino-N calibration of NIR scanning Jeffco/Carver extracted juice from clean cane

Ninety five Jeffco/Carver extracted juice samples (see 4.1.4.2) collected from hand cut experimental cane plots in 1996 from Bli Bli, Grafton, Macknade, Mackay and Yandina had amino-N determined via the ninhydrin method adapted from Amato and Ladd (1988) as described by Keating *et al.* (1999). These samples were filtered (0.8 μm) and scanned with a NIRSystems Model 6500 reflectance spectrophotometer fitted with a 0.5mm quartz cuvette for juice presentation scanning in the range of 600 to 2500 nm. The method used for NIR calibration was similar to those described by Brotherton and Berding (1995). Results of the calibration are presented in section 5.1.1.1.

4.1.2 Amino-N calibration of NIR scanning unfiltered first expressed juice

Sixteen first expressed juice samples were collected in duplicate from Mossman and Mulgrave mill juice labs during 2000 and immediately frozen. Each sample was analysed for amino-N via the ninhydrin method (Keating *et al.* 1999). The duplicate sample was scanned in transmission mode with a Foss-NIRSystems model 6500 with a beverage module set at a path length of 1mm scanning 400-2500nm as described in Appendix 26.

The NIR spectra wavelengths used were reduced to remove noise created by known water peaks and math treatments and scatter corrections applied to enhance spectral information (see Appendix 26). The calibration for amino-nitrogen was developed using partial least squares regression. Results are presented in section 5.1.1.2.

4.1.3 Asparagine calibration of NIR scanning unfiltered and filtered first expressed juice

During season 2000 twenty samples of first expressed juice from Mosman mill were collected in duplicate and frozen immediately. Each sample was analysed to determine asparagine concentration via High Performance Liquid Chromatography (HPLC) as described in Appendix 33. Unfiltered first expressed juice samples were scanned using a Cropscan 2000G NIR analyser in a 30mm pathlength liquid cell, between 720 and 1100nm. Samples were then centrifuged at 5000rpm for 15 minutes. The supernatant was decanted from the precipitate and the samples were rescanned in the same way as the unfiltered samples. Results are presented in section 5.1.1.3 and Appendix 27.

4.1.4 Amino-N calibration of NIR scanning prepared cane

Universal monitoring of all rakes of cane in a mill region via NIR became possible with the installation of the CAS NIR at Mulgrave mill in 1998 and Mossman, South Johnstone and Tully mills in 1999. The system, described by Staunton *et al.* (1999), is based on a NIR Systems 6500 scanning monochromator attached to the feed chute of number one mill where it scans prepared cane fibre. The instrument takes separate spectra on each sugarcane rake unit and is matched to the mill cane payment and process control computers.

4.1.4.1 Calibration development and validation on amino-N determined on first expressed juice

First expressed juice is collected in the mill juice lab and represents a sample of juice corresponding to a single rake unit. Samples of first expressed juice with their corresponding rake number identified were collected in duplicate and immediately frozen. These samples were analysed for amino-N via the ninhydrin reactive method described by Keating *et al.* (1999). Samples were collected over time to account for variation inherent in cane supply. Details of first expressed juice sample collection for calibration development of CAS NIR is presented in Table 1.

Table 1: First expressed juice samples collected for calibration of CAS NIR.

Year	Mill	Samples	Samples with NIR scans	Varieties	Classes	Farms	Weeks
1998	Mulgrave	302	265	15	7	163	19
1999	Mossman	109	105	15	11	75	10
	Mulgrave	119	115	15	7	78	15
	South Johnstone	28	26	21	6	22	5
	Tully	50	45	15	6	37	8
	Sub-total 1999	**306**	**291**				
2000	Mossman	286	278	17	7	89	12
	Mulgrave	331	318	24	7	142	8
	Sub-total 2000	**617**	**596**				
	Total	**1225**	**1152**				

Calibration development, described by Staunton *et al.* (1999), involved math treatment of spectral data including scatter correction, derivative mathematic application, wavelength region targeting and maximum allowable factor setting. Know wavelength regions that produce noise, such as spectral water peaks, were avoided. Calibration equations are 'global', meaning the location and specific conditions the NIR scanning head is exposed to does not influence the

output of the equation. A single global calibration equation for each separate component analysed by NIR is applied to CAS NIR units in all mills.

Developed calibration equations were initially validated 'on themselves' using redundant spectral data not used in the calibration equation itself. The predicted spectral result is compared with the laboratory result and plotted in a scatter plot. Linear trendlines are plotted (y = ax + b) and a correlation of the trendline (r^2) determined to evaluate the equation performance.

Calibrations are then tested for validation with data from a spectral dataset that does not include spectra used in the development of the calibration (often from the subsequent season). Trendlines and correlations are determined to evaluate the calibration as above. Results of calibration development and evaluation for amino-N using first expressed juice are presented in section 5.1.2.1.

4.1.4.2 Calibration development and validation on amino-N determined on Jeffco/Carver extracted juice

A sample of shredded cane fibre was compiled for a single rake by taking many 'snap' samples from across the entire rake of cane, sampled prior to the fibre entering number one mill. Each sample was thoroughly mixed, sub sampled for cutter grinding and mixed again. A single 500g ±0.5g sample of cutter ground fibre was placed in a steel cylinder cage and juice extracted by applying 10 000kg of hydraulic pressure for 60 seconds using a Carver Press (adapted from Muchow *et al.* 1993). These juice samples were analysed for amino-N via the ninhydrin reactive method as described above.

The calibration and validation process of CAS NIR on 'Jeffco/Carver' extracted juice was exactly as described above for first expressed juice, with results presented in 5.1.2.2.

Corresponding samples of first expressed juice were sampled from each rake where a Jeffco/Carver extraction was taken and amino-N determined via the ninhydrin technique.

4.1.4.3 Calibration development and validation on amino-N determined from methanol extraction

Samples of cane fibre collected as described in 4.1.4.2 were taken at Mossman and Mulgrave mills during season 2002 and frozen. After collection in the mills the collected fibre was stored at -20°C until it was defrosted for extraction. Soluble amino acids were extracted from cane fibre by adding 4g FW cane fibre in 20mL 100% methanol with 10μM nor-Leucine as an internal standard (1:5 extraction). The methanol/fibre samples were incubated for at least 48 hours at 4°C and then stored at -20°C until samples were assayed.

The amino-N of the extract was determined by the ninhydrin technique as described by Keating *et al.* (1999). CAS NIR amino-N calibration and validation was conducted on a dataset of methanol extracted amino-N from Mossman 2002 as described in 4.1.4.1. Results of CAS NIR calibration and validation for methanol extracted amino-N are presented in 5.1.2.3.

4.1.5 Total N calibration of NIR scanning prepared cane

Samples of cane fibre collected as described in 4.1.4.2 were taken at Mossman and Mulgrave mills during season 2002 and frozen. Total N was determined by mass spectrometry. Samples were oven dried (50ºC), finely ground and analysed by continuous-flow automated nitrogen and carbon (ANCA) mass spectrometry (Tracer Mass, Europa Scientific, Crewe, UK).

The total N of samples from Mossman mill was used in calibration development and validation using the same technique used to calibrate for amino-N (4.1.4.1). Results of CAS NIR calibration and validation for total N are presented in 5.1.2.4.

4.1.6 Data transformation of amino-N or total N values prior to NIR calibration development

Non-linear data transformations are a statistical tool used to produce population distributions that are more symmetrical, and hence more like normal curves (Hogg and Craig 1978). Different transformation conversions have varying ability to transform a dataset into a 'normal' distribution. The right skewed amino-N data is log transformed to produce a more 'normal' population and then calibration development attempted. The output of the calibration equation has the anti-log applied to return the data to the original units. Calibration development occurs as described in 4.1.4.1. Results of log transformation of amino-N data from Jeffco/Carver extracted juice from Mossman 2002 and subsequent CAS NIR development and validation are presented in 5.1.2.5.

4.1.7 Comparison of amino-N determined on first expressed juice and Jeffco/Carver extracted juice

In the 2002 season 150 samples of fibre from Mossman and 143 from Mulgrave mill were collected and had juice extracted via Jeffco/Carver as described in 4.1.4.2. These samples had amino-N determined via the ninhydrin method (Keating *et al.* 1999). These samples, matched to a specific mill rake, had the corresponding first expressed juice samples collected concurrently. The amino-N of these first expressed juice samples was also determined via the ninhydrin technique. A comparison between the amino-N values derived from Jeffco/Carver and first expressed juice samples is presented in 5.1.3.

4.1.8 Investigation of impact of hydraulic pressure and cane fibre preparation on extraction of amino-N

During season 2000 14 rakes from Mossman mill were sampled to determine the impact of hydraulic pressure and extent of cane preparation on extraction of amino-N. Samples of cane fibre were sampled as described in 4.1.4.2 with the sample divided in two with half the sample being cutter ground and half remaining as mill shredded fibre.

Each sub sample (cutter ground or shredded) was prepared in duplicate for juice extraction using the Carver Press as described in 4.1.4.2. One of the duplicate samples received a hydraulic pressure of 10 000kg for 60 seconds (heavy hydraulic pressure) (standard extraction process, section 4.1.4.2) and the other duplicate sample was subjected to hydraulic pressure from the Carver Press until juice started to flow from the steel cylinder cage (light hydraulic

pressure), at which point hydraulic pressure ceased. Effect of hydraulic pressure (light or heavy) at each level of cane preparation was plotted and effect of cane preparation (shredded or cutter ground) at each hydraulic pressure of juice extraction was plotted and presented in 5.1.4.

4.1.9 Investigation of laboratory determination of amino-N

An investigation into the performance of the laboratory technique used to determination amino-N levels in sugarcane juice is warranted to ensure this is not a source of variation that could reduce the ability of CAS NIR to predict amino-N.

4.1.9.1 Investigation of effect of juice filtering prior to ninhydrin assay

As part of the ninhydrin assay process (Keating *et al.* 1999) juice is prefiltered through a Millipore 'AP20' glass fibre membrane prior to filtering thorough a 0.8μm filter (Millipore 0.8μm mixed cellulose ester membrane). Prefiltering reduces much of the colloid load that would block the 0.8μm filter. To determine if filtering through the 0.8μm filter has any effect on amino-N, such as binding amino acids or proteins to the filter, six samples of Jeffco/Carver extracted juice from the dataset sample described in 4.1.7 were subjected to either the conventional assay procedure of including the 0.8μm filtering, or had the 0.8μm filtering step omitted. Results are presented in section 5.1.5.1.

4.1.9.2 High Performance Liquid Chromatograph (HPLC) determination of amino-N

During season 2001 82 samples of Jeffco/Carver extracted juice collected from Mulgrave mill were analysed for individual amino acids via HPLC. These samples were chosen because they had corresponding CAS NIR spectra. Analysis used a pre-column derivitisation with phenylisothiocyanate method based on the optimum method of Vasanitis and Molnar-Perl (1999), using an Alltech Alltima C18 column 4.6mm x 150mm. This system measured the concentration of each of 19 amino acids (Alanine, Arginine, Asparagine, Aspartic acid, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, and Valine). However, recovery issues with methionine, threonine, aspartic acid, proline, glycine, and glutamine meant the concentrations of these individual amino acids must be used with caution. However, as their individual concentrations are small, the impact on the combined amino-N pool is within experimental error (10%) these individual amino acids were included in the total amino-N pool. Methionine, however, was excluded from all calculations because of its extremely high values (due to ammonium co-eluting with the methionine). All other 18 measured amino acids were used on all calculations described here.

Amino acid concentration is in the units of nmol/ml juice. The number of nitrogen atoms associated with each amino acid measured is known (Table 2). To determine amino-N (in ng/ml) the sum of the total number of known nitrogen atoms in a sample is calculated and multiplied by the atomic weight of nitrogen (MW_N=14). The result is divided by 1000 to establish amino-N in μg/ml.

Table 2: Number of nitrogen atoms associated with HPLC measured amino acids.

Amino acid	No N atoms	Amino acid	No N atoms	Amino acid	No N atoms
Alanine	1	Glycine	1	Proline	1
Arginine	4	Histidine	3	Serine	1
Asparagine	2	Isoleucine	1	Threonine	1
Aspartic acid	1	Leucine	1	Tryptophan	2
Glutamic acid	1	Lysine	2	Tyrosine	1
Glutamine	2	Phenylalanine	1	Valine	1

4.1.9.3 Investigation into validity of the ninhydrin assay

The ninhydrin reactive test is based on the reaction of ninhydrin with the α-amino N of the amino acids to produce a blue-violet colour which is detected by spectrophotometer (540 nm). A standard plot converting absorbance to concentration of ninhydrin reactive N (nmol/ml) is constructed from asparagine standards.

Summing the concentration of individual HPLC detected amino acids (nmol/ml) produces a total concentration of all measured amino acids.

Ninhydrin reactive N (measuring amino acid concentration in nmol/ml) is plotted against HPLC amino acid concentration (nmol/ml) to evaluate the performance of the ninhydrin reactive test and is presented in 5.1.5.2.

4.1.9.4 Investigation into the amino-N conversion equation

To convert ninhydrin reactive amino acids (nmol amino acid/ml) to amino-N the ninhydrin reactive N is divided by 1000 (to μmol amino acid/ml) and multiplied by the atomic weight of nitrogen (MW_N=14) to yield μg α-amino-N/ml. A previously determined conversion equation (reported in Keating *et al.* 1999) converts μg α-amino-N/ml to amino-N (μg/ml). The results of ninhydrin determined amino-N is plotted against the result of HPLC determined amino-N to evaluate this conversion equation and presented in 5.1.5.3.

A new conversion equation was developed from this dataset by plotting the ninhydrin reactive α-amino-N against the HPLC determined amino-N (μg/ml). The equation of the trendline of this plot is the conversion equation predicted on this dataset for converting ninhydrin reactive α-amino-N to ninhydrin determined amino-N (see Figure 19 in 5.1.5.3).

4.2 Objective 2: Develop a communication plan identifying the key steps necessary for successfully implementing a nitrogen monitoring technique to an entire mill region

4.2.1 Framework used for development of communication plan

To develop a communication plan that can implement the amino-N monitoring technique to an entire mill district a framework described by Foster (2000) was followed. The framework outlines the principles to follow as:

1. Conduct a situation analysis
2. Set the objectives
3. Establish the learning principles
4. Describe the level of participation
5. Create the process (metaprocesses and microprocesses)
6. Develop the resources
7. Implement the process
8. Evaluate the process

The communication plan development is presented in Appendix 15.

4.2.2 Interpretation of amino-N

Six experimental nitrogen input trials where sugar yield and amino nitrogen was determined were used to plot a diagnostic relationship between amino-N and relative sugar yield. Relative sugar yield is presented as a fraction of one when one equals sugar yield when nitrogen was non limiting. Experimental details of the trials used are provided in Keating *et al.* (1999) and Keating *et al.* (2000). The diagnostic relationship is presented in 5.2.2.1.

The diagnostic yield response plot was examined 'by-sight' and the amino-N value which best corresponds to the value below which the vast majority of relative sugar yields are below 1 was estimated and used as the upper amino-N value of the 'low amino-N range'. The amino-N value above which all relative sugar yields are equal to one (except in a suspected toxicity case) was determined as the value which amino-N above this is 'excess'. The target range for amino-N is the values between the low and excess values. The target range is divided evenly to produce 'low target' and 'high target' ranges. A summary table of the proposed amino-N interpretation is presented in 5.2.2.1.

4.2.3 Development of 'Guidelines for Cane Farmers on use of Amino Nitrogen Data'

Two 'Grower Working Groups' were established at the commencement of the project to provide a mechanism for participatory research into implementation of the amino-N monitoring technique. These groups were established in the Mossman and Mulgrave mill areas. These groups consist of 15 growers in Mulgrave and 7 in Mossman with a local productivity officer participating in each group. Amino-N data was presented to these growers early in the project and a wide range of presentation formats and data interpretations investigated for ease of use by growers. In consultation with growers from both groups a document titled 'Guidelines for Cane Farmers on use of Amino Nitrogen' was produced. The document is in a *Frequently Asked Questions* format using questions growers in the working groups thought farmers would want to know about amino-N. The interpretation of amino-N for farmers was described and

presented as being a balance between N supply and N demand to the grower working groups. Growers requested that this interpretation be included in the guidelines document. The 'Guidelines for Cane Farmers on use of Amino Nitrogen' is presented in Appendix 29.

4.2.4 In-season reporting of amino-N

Amino-N data from CAS NIR is recorded on every rake of cane entering the mill. To provide growers with rake amino-N data during the season the mills rake reporting mechanism should be utilised. The mill provides regular feedback of cane payment details to growers. Amino-N descriptions (low, low target, high target, excess) can be added to this feedback mechanism. If the mill chooses not to add amino-N data with rake feedback, rake amino-N data should be provided at regular intervals through the season, such as monthly or at the conclusion of each harvesting round. Section 5.5.2.2 presents an example of in-season reporting of amino-N.

4.2.5 End-of-season reporting of amino-N

End-of-season amino-N reporting is on the block level and not the rake level as with in-season reporting. Rake amino-N data needs to be converted to block amino-N values. Each block of cane often consists of greater than one rake of cane. To arrive at a single block amino-N value each rake amino-N value for a single block is weighted to represent the contribution that rake (R) makes to the block using the following formula:

$$\text{Amino-N of block} = \frac{(\text{tonnes R1} \times \text{amino-N R1}) + \ldots (\text{tonnes Rn} \times \text{amino-N Rn})}{\Sigma \text{ tonnes R1}\ldots\text{Rn}} \quad \text{Eqn 1}$$

The block amino-N value is then converted to the amino-N description (low, low target, high target, excess) and reported with basic block data (block, variety, class, CCS).

Thematic maps are also produced when feeding information back to growers for end-of-season amino-N reporting. Thematic maps are colour coded to show different amino-N levels. Mill productivity departments routinely produce an end-of-season block productivity report which often includes a farm map. End-of-season amino-N reporting should accompany routine block productivity reports.

4.3 Objective 3: Identify relationships between crop N status (as determined by laboratory based amino-N assays) and CCS at the block scale

Relationships between amino-N (representing crop N status) and CCS are determined by plotting scatter relationships. Only data from laboratory determined amino-N is used in analysing these relationships due to the lack of robust equations for NIR. Scatter plots trendlines are used to evaluate the relationship.

All data collected during the 2000 crushing season is evaluated. Relationships are assessed by drilling down through the data to remove factors that may influence CCS apart from the nitrogen status of the crop. Relationships were

only evaluated on units with three or more data points. The ideal relationship assessment would be made on rakes sampled from a single block of cane. A single block of cane is assumed to be a homogenous unit with the same variety, class, harvesting date, crop age, and fertiliser regime present. Unfortunately the limited dataset did not yield single blocks with greater than 3 rakes of cane analysed.

4.4 Objective 4: Explore the impact of the different components of cane supply (sound cane, suckers and extraneous matter) on the amino-N monitoring technique

For the overall experimental layout and treatment application see Dart et al (2000).

The investigation into the effect of extraneous matter on the amino-N monitoring technique was carried out on plant cane harvested in September 1997. The 0 and 180 kg N ha^{-1} treatments were sampled but only samples from replicates 1 and 2 were analysed due to replicate 3 not growing well in the plant crop. Hand sampling of stalks from plots were conducted as presented in Dart et al. (2000). In addition, 1 to 1.5 kg samples of surface trash, green leaf, cabbage, and surface soil were collected from the treatment plots. Samples were transported to Brisbane for sample preparation and juice extraction.

For juice extraction all collected samples were ground through Jeffco cutter grinder. Juice was extraction was by Carver hydraulic press based on methods outlined in Muchow et al. (1993).

Extraneous matter was added at either 2% or 5% by weight in conjunction with millable stalk, no multiple additions were made. Each of the trash, green leaf, cabbage or soil was added at the two rates with 1kg of millable stalk and the combined sample well mixed. Due to the low density of the trash material it was not possible to pack 500g of mixed sample into the press. For 2% EM addition a 340g sample was used and for 5% EM addition a 230g sample was used. The pre and post pressing weights for the fibre were recorded to give an indication of the extraction efficiency.

The collected juice samples were analysed for amino-N by both ninhydrin assay and HPLC according to the methods set out in Keating et al. (1999). Juice samples were also analysed for total N by the Kjeldahl method.

Results are presented in section 5.4.

5. Results and Discussion

5.1 Objective 1: Develop and validate mill scale calibration of NIR as a practical tool in routine monitoring of amino-N or total N levels in cane supply

For the amino-N monitoring technique to be implemented at a mill region scale a rapid, reliable and inexpensive technique for determining the amino-N of every block of cane in the mill region is needed. All cane is processed through a central mill, making the mill an ideal location to measure amino-N. NIR monitoring systems based at the mill capable of measuring amino-N could provide the monitoring tool sought for amino-N.

5.1.1 NIR calibration using cane juice spectra

Projects CSC21s and CTA029 routinely conducted amino-N assays on juice samples extracted from carver pressing fibrated cane (Keating *et al.* 1999) from which the basic N response curves were constructed. Results of attempts to further this response curve to calibrate NIR systems for amino-N in cane juice are presented.

5.1.1.1 Amino-N calibration of NIR scanning Jeffco/Carver extracted filtered juice from hand sampled cane

Sugarcane juice was extracted from hand cut cane fibrated through a Jeffco cutter grinder and carver pressed. An experimental beverage analyser NIR was

calibrated on 95 samples of juice ranging in amino-N from 19 to 666 μg amino-N/ml juice. The NIR calibration equation validation was conducted on the same dataset and is presented in Figure 1.

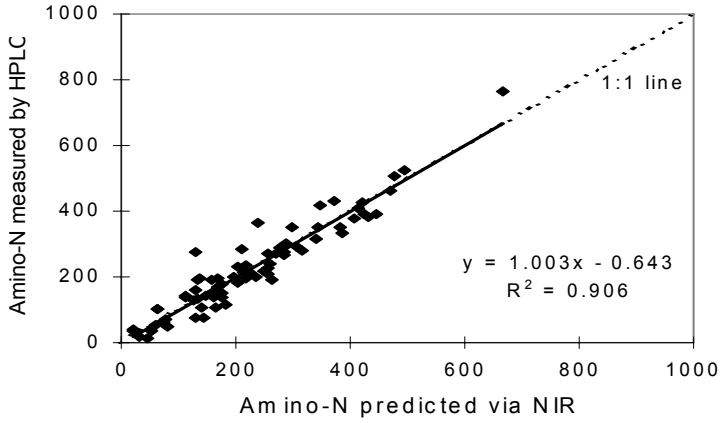


Figure 1: Validation plot of NIR calibration using reflectance NIR on clean cane juice.

The NIR validation plot shown in Figure 1 is a good calibration curve that would suit the purposes of this project. It is impractical (at this stage) to perform on-line NIR scans on cane juice as clean as was conducted in this instance on every rake of cane entering the mill. This calibration of amino-N in juice shows amino-N levels can be detected via reflectance NIR equipment and adequate calibrations are possible, even though the amino acids are present at only low concentrations in the juice. The challenge is to develop an NIR calibration for amino-N that can be applied universally in the sugar industry.

5.1.1.2 Amino-N calibration of NIR scanning unfiltered first expressed juice

Sixteen unfiltered first expressed juice samples from Mossman and Mulgrave mills, collected during 2000, were analysed via an experimental transmission NIR (Foss-NIRSystems model 6500) with a beverage module set at a pathlength of 1mm. Figure 2 shows the validation plot for this calibration attempt.

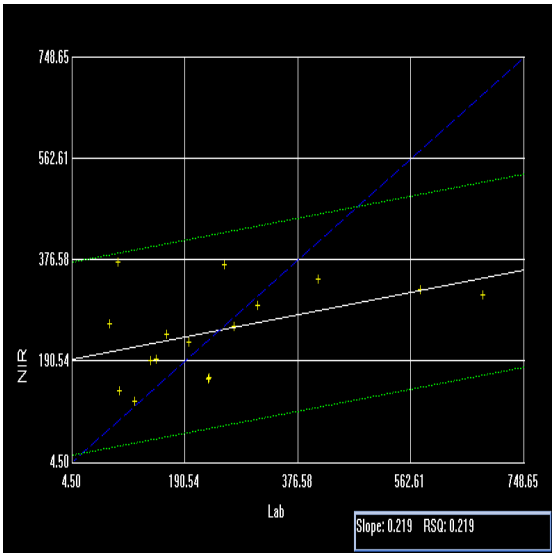


Figure 2: Validation plot of NIR calibration of unfiltered first expressed juice samples.

Figure 2 shows calibration of NIR of unfiltered first expressed juice samples was not possible using this NIR calibration technique. Reasons given by the researchers who conducted this research were that the amino-N concentrations in juice were too low to be detected by NIR, in apparent conflict with what is reported in 5.1.1.1. The complete report is attached in Appendix 26.

5.1.1.3 Asparagine calibration of NIR scanning unfiltered and filtered first expressed juice

Twenty samples of first expressed juice with a known asparagine concentration were analysed by a liquid cell NIR system and a calibration for asparagine attempted. The concentration of asparagine used in the calibration attempt

ranged from 2μmol/ml to 26μmol/ml. Attempts at calibrating for asparagine in unfiltered first expressed juice show no calibration was possible (Appendix 27).

The twenty juice samples were then centrifuged and the supernatant decanted from the precipitate and the samples were scanned in the same way as the unfiltered samples. The resulting calibration attempt on the centrifuged samples produced a correlation of 0.8758, indicating NIR has the ability to identify concentrations of asparagine in this range. The researchers suggested the colloid load of first expressed juice interfered with the ability of the beverage analyser NIR to calibrate for asparagine. A full report of the calibration analysis of asparagine is presented in Appendix 27.

5.1.1.4 Conclusions from NIR calibration using juice spectra

The calibration correlations presented above clearly indicate that if cane juice were to be scanned by NIR for amino-N determination the juice would require filtering prior to scanning. NIR has the ability to measure the low concentrations of amino-N routinely detected in sugarcane juice, with sample presentation to the scanning instrument having a significant influence on the ability of NIR to calibrate. Berding (*pers comm*) supports this finding that suggested the ability of NIR to measure pol and brix with an experimental beverage analyser NIR was greatly enhanced by the filtering of the juice prior to scanning.

5.1.2 Cane Analysis System NIR

During 1998 and 1999 the Sugar North mills (Mossman, Mulgrave, South Johnstone and Tully) installed Cane Analysis System (CAS) NIR systems (Staunton et al. 1999) scanning the sugarcane fibre prior to it entering number one mill. This installation of NIR to the milling train provided an opportunity of monitoring all cane in a mill region for amino-N if the CAS NIR could be calibrated for amino-N. Since 1999 the South Johnstone NIR system has been de-commissioned, leaving Mossman, Mulgrave and Tully mills with operating CAS NIR systems.

5.1.2.1 Calibration development and validation of amino-N determined on first expressed juice

As first expressed juice is relatively straightforward to collect, development of a calibration equation based on amino-N determined on first expressed juice would represent the easiest outcome for validation purposes. This formed the initial aims for the NIR component of this project.

Three calibration equations were attempted using first expressed juice. Each calibration equation used a progressively larger dataset to account for the variability that was believed to be responsible for inadequate calibrations.

- Equation 1 was developed from data collected during 1998 from Mulgrave mill. Milestone Reports 2 and 3 (Appendices 19 and 20) report on this equation.
- Equation 2 was developed from data collected during the 1999 crush from Mossman, Mulgrave, South Johnstone and Tully mills. Milestone Reports 3 and 5 (Appendices 20 and 22) report on this equation.
- Equation 3 was developed from data collected during the 1998, 1999 and 2000 crushing seasons. Milestone Report 5 (Appendix 22) reports on this equation.

5.1.2.1.1 Equation 1 calibration equation

Two hundred and sixty five samples of first expressed juice collected from Mulgrave mill during 1998 had successful CAS NIR scans associated with them. The samples had amino-N determined via ninhydrin and these values were used to calibrate the initial amino-N CAS NIR calibration equation. Table 3 presents the validation data for this calibration attempt when redundant spectral data (data from the calibrating dataset not used in the actual calibration) from 1998 was used for validation and when 1999 amino-N data was used in validation.

Table 3: Performance of Equation 1 amino-N CAS NIR calibration equation developed from first expressed juice.

Equation	Data used to build	Validation Dataset	Trendline	Correlation
Equation 1	Mulgrave 1998	Mulgrave 1998		$r^2 = 0.45$
		Mossman 1999	y = 0.3066x + 116.89	$r^2 = 0.26$
		Mulgrave 1999	y = 0.2429x + 171.91	$r^2 = 0.15$
		Tully 1999	y = 0.1545x + 170.24	$r^2 = 0.05$

Validation of the initial amino-N calibration equation (Equation 1) showed a correlation of $r^2 = 0.45$ when samples of first expressed juice from 1998 were used. The calibration validation, while unusable, suggested CAS NIR is able to pick up trends in amino-N and further calibration development could produce a more robust equation.

When first expressed juice samples from 1999 were used to validate the equation the calibration did not validate well. Of particular note is that the slope of the trendlines (a where y = ax + b) is well below 1, indicating a very flat calibration (see Appendix 5). These very flat validations indicate that as amino-N values increase to high levels (as determined by ninhydrin), NIR under predicts the amino-N value by an increasing amount. This poor validation indicates Equation 1 does not accurately estimate amino-N values and a new equation should be developed.

5.1.2.1.2 Equation 2 calibration equation

Samples collected from Mossman, Mulgrave, South Johnstone and Tully during 1999 were used to develop a new calibration equation for amino-N (Equation 2). Table 4 presents the validation performance of this calibration equation when redundant spectral data from is used as the validating dataset, and when validation data from season 2000 is used.

When 1999 data is used to validate Equation 2 the results vary greatly between the mills. The Mossman and South Johnstone validation datasets indicate there

is potential that a robust equation for amino-N can be made from first expressed juice. The trendline slopes of the validations are still very low, indicating CAS NIR is still unable to accurately estimate high amino-N values (see Appendix 6).

When first expressed juice samples collected from the 2000 season were used as the validating dataset the calibration did not hold (Appendix 12) indicating variations introduced in the season 2000 dataset were not accounted for in the equation. A new calibration that could account for these variations was needed.

Table 4: Performance of Equation 2 amino-N CAS NIR calibration equation developed from first expressed juice.

Equation	Data used to build	Validation Dataset	Trendline	Correlation
Equation 2	Mossman 1999 Mulgrave 1999 South Johnstone 1999 Tully 1999	Mossman 1999	y = 0.5187x + 119.63	r^2 = 0.41
		Mulgrave 1999	y = 0.2144x + 146.89	r^2 = 0.06
		South Johnstone 1999	y = 0.3084x + 105.3	r^2 = 0.57
		Tully 1999	y = 0.2876x + 102.66	r^2 = 0.28
		Mossman 2000	y = 0.3975x + 120.52	r^2 = 0.18
		Mulgrave 2000	y = 0.0908x + 157.52	r^2 = 0.01

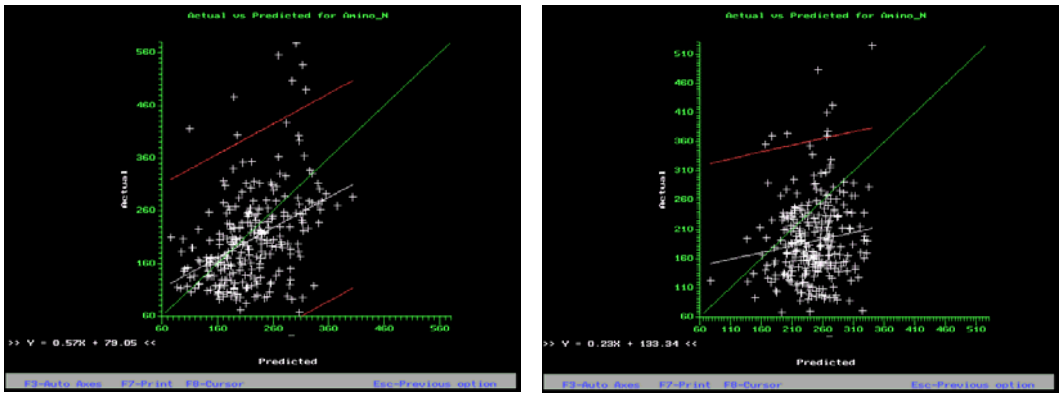
5.1.2.1.3 Equation 3 calibration equation

A new calibration equation using all of the first expressed juice samples collected from 1998, 1999 and 2000 was used to develop a global equation for amino-N. It was hoped the variability inherent at each mill over the three seasons could be accounted for in the equation. Table 5 and Figure 3 presents the validation performance of this equation (see Appendix 13).

The calibration validation of equation 3 was poor. Equation 3 was developed from three mills across three seasons, which should have accounted for the inherent variability associated with sampling cane for NIR calibration. The fact a validation was not possible strongly suggests a calibration based on amino-N determined on first expressed juice is not achievable.

Table 5: Performance of Equation 3 amino-N CAS NIR calibration equation developed from first expressed juice.

Equation	Data used to build	Validation Dataset	Trendline	Correlation
Equation 3	Mulgrave 1998 Mossman 1999 Mulgrave 1999 Tully 1999 Mossman 2000 Mulgrave 2000	Mossman 2000	y = 0.57x + 79.05	$r^2 < 0.2$
		Mulgrave 2000	y = 0.23x + 133.34	$r^2 < 0.2$



(a) (b)

Figure 3: Validation of Equation 3 calibration equation using first expressed juice samples from Mossman (a) and Mulgrave (b) collected during the 2000 crush. Laboratory determined amino-N (y axis) vs NIR predicted amino-N (x axis).

5.1.2.1.4 Summary of NIR calibration attempts for amino-N determined on first expressed juice

From amino-N concentrations determined on first expressed juice it has not been possible to develop a satisfactory calibration using the CAS NIR. After the Equation 3 calibration was attempted and proved unable to estimate amino-N a decision was made that further calibration attempts using amino-N determined on first expressed juice should not be pursued.

The project team deemed the potential value of having a usable amino-N calibration for the CAS NIR system was attractive enough to investigate reasons behind why CAS NIR could not be calibrated for amino-N and if an alternative method was available for developing a calibration.

5.1.2.2 Calibration development and validation of amino-N determined on Jeffco/Carver extracted juice

A possible reason CAS NIR is unable to develop a robust calibration equation for amino-N determined on first expressed juice is that the 1st mill does not extract all of the amino-N present in the cane stems and that it extracts a variable concentration of amino-N present. This can mean that first expressed juice amino-N is not representative of the amino-N being scanned by the CAS NIR (see section 5.1.3). To account for this variability a standard juice extraction technique utilising the Jeffco cutter grinder and Carver press and sampling shredded fibre from the mill feed chute was used to obtain the juice sample. The amino-N concentration was measured by ninhydrin and used in calibration development. Figure 4 presents a calibration from Jeffco/Carver determined amino-N and is validated using redundant spectral data not used in calibration from the same dataset.

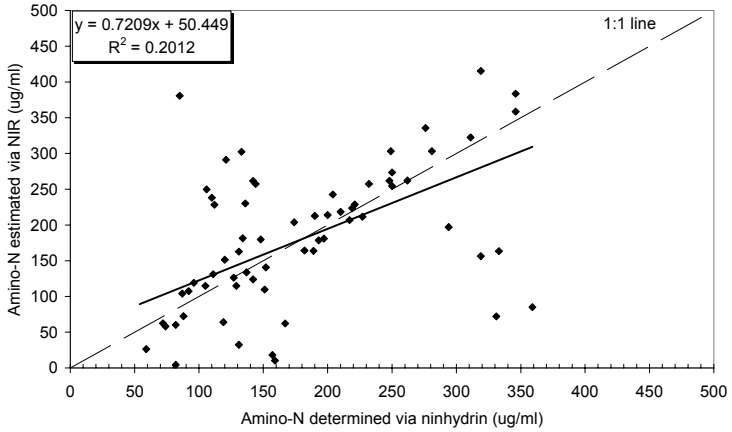


Figure 4: Amino-N calibration equation validation based on Jeffco/Carver extracted juice, Mulgrave 2001.

The plot presented in Figure 4 does not have a correlation greater than the validation plots for calibration equations developed using first expressed juice. The plot does however have a 'core' of data close to the 1:1 line, indicating CAS NIR is predicting a number of samples accurately. The slope of the trendline is closer to 1 than was found in calibrations developed on first expressed juice amino-N (Appendix 5) indicating the calibration is better at predicting higher amino-N values.

Further sampling of Jeffco/Carver samples occurred during season 2002 from Mossman (150 samples), Mulgrave (143 samples) and Tully (63 samples) mills. This data was combined with the 2001 data and a global calibration developed. Figure 5 presents the validation of this calibration when redundant spectral data is used as the validating dataset.

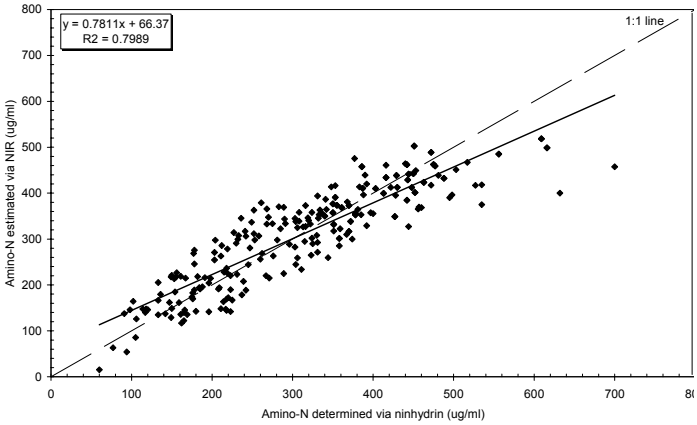


Figure 5: Amino-N calibration equation validation based on Jeffco/Carver extracted juice, Mulgrave 2001, Mossman 2002, Mulgrave 2002, Tully 2002.

The global calibration equation presented in Figure 5 is promising, with a slope much closer to 1 (0.7811) than has been previously possible with first expressed juice, and a better correlation (r^2=0.7989). This calibration needs to have a validating dataset predicted to test the equation. Further improvements with this calibration should occur as more data is added from future seasons.

5.1.2.3 Calibration development and validation of amino-N determined from methanol extraction

Methanol extraction of amino-N theoretically extracts all amino acids from the sample through breaking down of all cell walls in the fibre sample. When ninhydrin amino-N is determined on methanol extracted juice the result is theoretically the absolute amino-N of the sample. An attempt was made to calibrate CAS NIR using the results of methanol extracted amino-N and is presented in Figure 6.

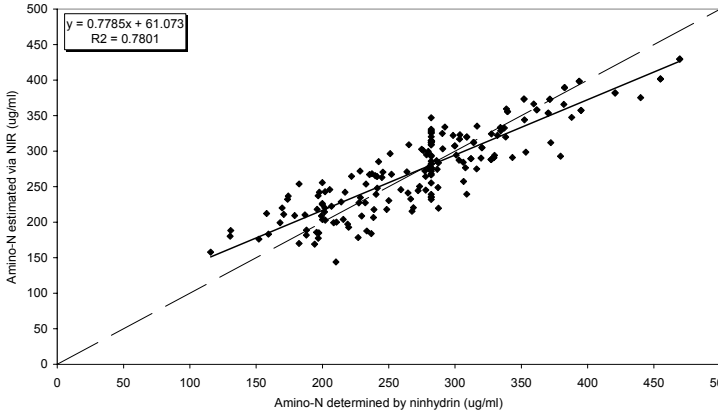


Figure 6: Amino-N calibration equation validation based on methanol extracted juice, Mulgrave 2001, Mossman 2002, Mulgrave 2002, Tully 2002.

The calibration presented for methanol extracted amino-N in Figure 6 was validated using redundant spectra not used in the calibration itself from the calibrating dataset. The result of this calibration is very similar to the calibration achieved for Jeffco/Carver extracted juice presented in Figure 5. This indicates that Jeffco/Carver extracted amino-N is comparable with methanol extraction in its ability to calibrate NIR and also in the amount of amino-N extracted (data not shown). Jeffco/Carver extracted amino-N is representative of the absolute amino-N of the sample being scanned by NIR.

Potential reasons why a better calibration cannot be developed at present are not juice extraction based and are further investigated in section 5.1.5.

5.1.2.4 Calibration development and validation of total N

The total N of fibre samples taken from Mossman, Mulgrave and Tully mills in season 2000 was determined and an NIR calibration attempted. A total N calibration was developed as a 'fall back' position should a calibration for amino-N not be possible. Figure 7 presents the calibration validation of total N when redundant spectra from the calibrating dataset is used for validation of total N.

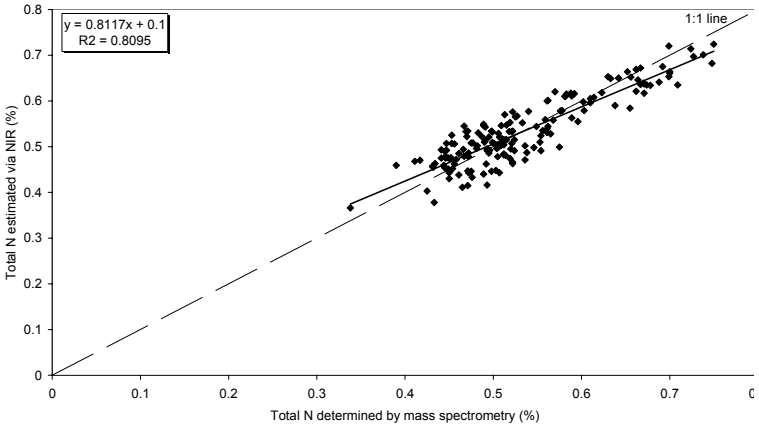


Figure 7: Total N calibration equation validation, Mossman 2002, Mulgrave 2002, Tully 2002.

The calibration validation for total N presented in Figure 7 needs to be validated using a dataset not used in the calibration development (such as from the 2003 season), however the r^2 of 0.8095 is promising. Once a robust validation from a non-calibration dataset is achieved, the equation is usable on all samples of cane scanned by NIR. The validating dataset can also be used to improve the calibration by adding data to the calibration development dataset. This process is ongoing.

5.1.2.5 Investigation of data transformation prior to NIR calibration development

Amino-N data sampled from mills is not normally distributed. Histograms have repeatedly shown the data is strongly skewed to the right (Figure 8).

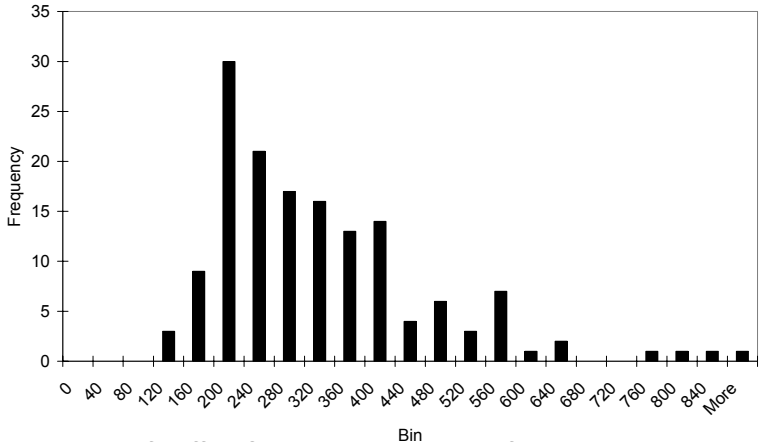


Figure 8: Histogram of Jeffco/Carver amino-N data from Mossman mill 2002.

NIR calibrations assume populations are normally distributed. In order to test if the lack of a normal distribution of amino-N is contributing to the inability of CAS NIR to calibrate a robust amino-N calibration equation the data is transformed and a new calibration attempted. The log of the ninhydrin amino-N values was taken to transform the data to a more normal distribution, as shown in Figure 9 (note the anti log was applied to the histogram bins).

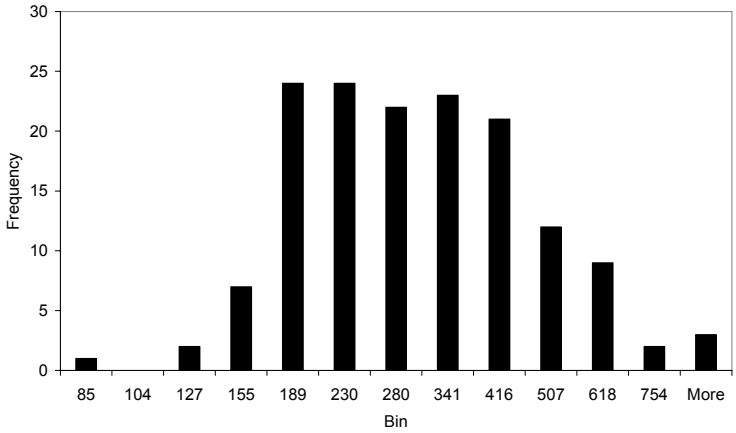


Figure 9: Histogram of Jeffco/Carver amino-N data from Mossman mill 2002 when a log (base 10) variance stabilising transformation is applied.

The transformed data is more normally distributed than the untransformed data. To test whether this data transformation contributes to improving the CAS NIR calibration for amino-N calibrations from the same dataset of untransformed and transformed data is compared in Figures 10 and 11. Validations are performed on the calibrations from redundant dataset spectra used to develop the calibrations.

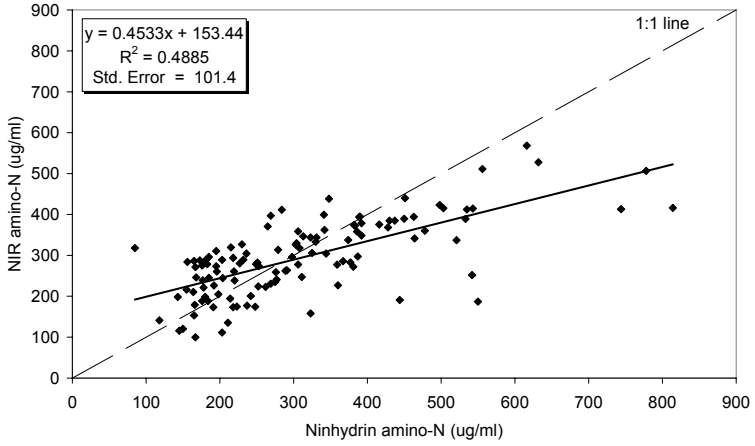


Figure 10: Amino-N calibration validation for Jeffco/Carver extracted juice from Mossman mill 2002.

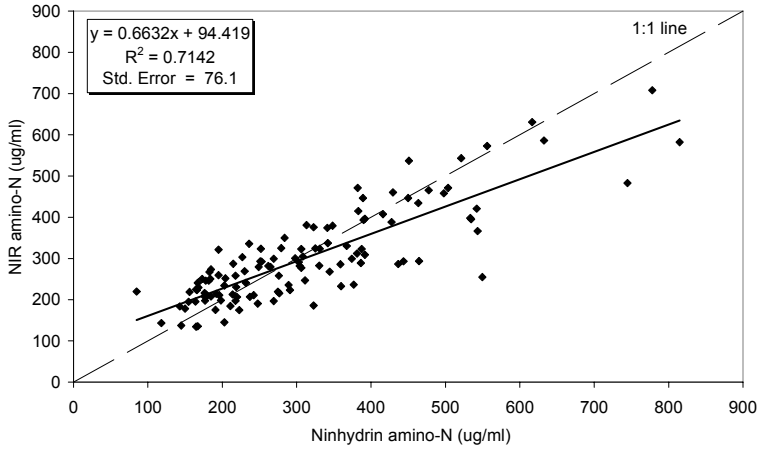


Figure 11: Amino-N calibration validation for Jeffco/Carver extracted juice from Mossman mill 2002, amino-N data log transformed for calibration.

The log transformation of amino-N data prior to calibration produces a better correlation when compared with data that has not been transformed (r^2 = 0.71, r^2 = 0.49 respectively). The slope of the fitted trendline is closer to one in the transformed data than the untransformed data (0.66, 0.45 respectively) indicating CAS NIR is better able to estimate high amino-N values when calibrated on a transformed dataset. Note that the validations presented in Figures 10 and 11 use redundant spectra from the calibration dataset not used in the calibration. Full validation of the equation should be undertaken with an entirely different dataset to establish equation robustness. Log data transformation is used on datasets prior to NIR calibration presented in sections 5.1.2.2, 5.1.2.3 and 5.1.2.4.

5.1.3 Comparison of amino-N determined on first expressed juice and Jeffco/Carver extracted juice

The CAS NIR scans fibrated cane as it enters number one mill. For a robust calibration to be developed laboratory determined amino-N values must

accurately represent the amino-N of the fibre that NIR is scanning. If first expressed juice does not accurately represent the amino-N of the total cane supply, CAS NIR may be unable to develop a calibration equation based on first expressed juice.

Figure 12 suggests that as the amino-N of juice extracted via Jeffco/Carver increases, the associated first expressed juice amino-N sample does not extract as much amino-N, as indicated by the slope of the trend line being less than 1 (0.7818).

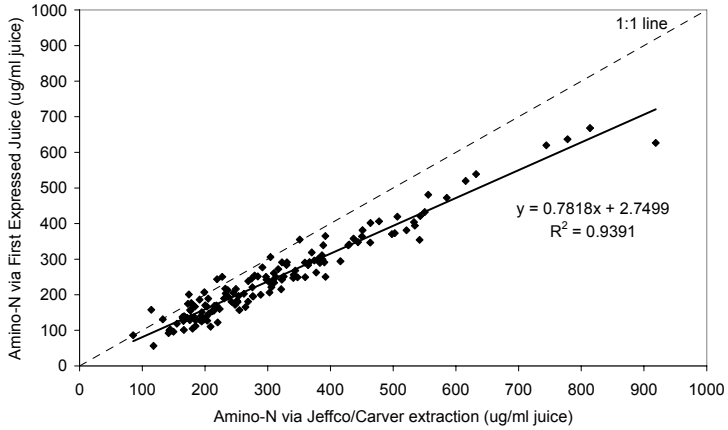


Figure 12: Amino-N in juice extracted via different methods, Mossman 2002.

Additionally, Figure 13 shows that the proportion of Jeffco/Carver amino-N extracted into first expressed juice has a greater variability at low values, suggesting the amino-N present in first expressed juice does not consistently reflect the amino-N present in the whole cane supply.

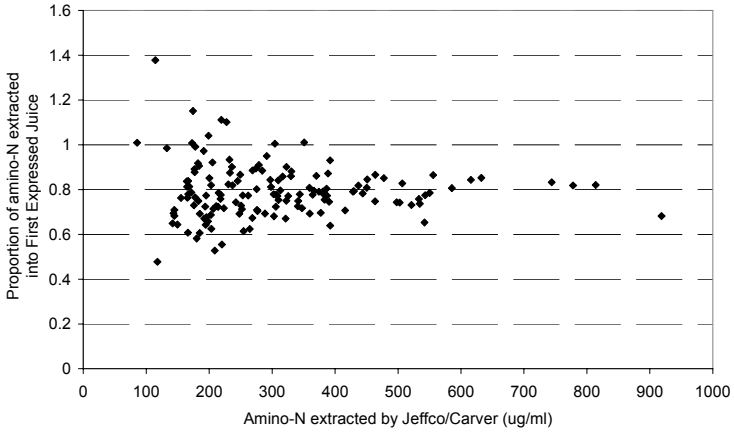


Figure 13: Proportion of Jeffco/Carver extracted amino-N extracted into first expressed juice, Mossman 2002.

5.1.4 Effect of hydraulic pressure and cane fibre preparation on extraction of amino-N

The apparent variation in amino-N extracted into first expressed juice when compared with Jeffco/Carver could come from two possible sources. First, the hydraulic pressure applied by the carver press is greater than is applied when extracting first expressed juice. Second, the level of fibration applied to the cane prior to juice extraction. The Jeffco cutter grinder 'cuts' the sample finer, possibly rupturing more cells prior to extraction via hydraulic pressure.

Figures 14 and 15 highlight that increasing the level of hydraulic pressure applied to sugarcane fibre does not change the extraction of amino-N, however increasing the cell rupture rate (by Jeffco cutter grinding) does increase amino-N extraction.

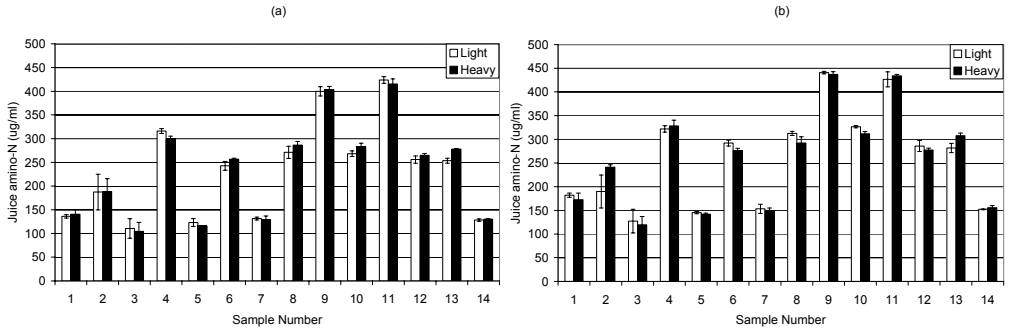


Figure 14: Extraction of amino-N from sugarcane fibre when either a light (□) or heavy (■) hydraulic press is applied to fibre that has been shredded (a) or fibre that has been Jeffco cutter ground (b).

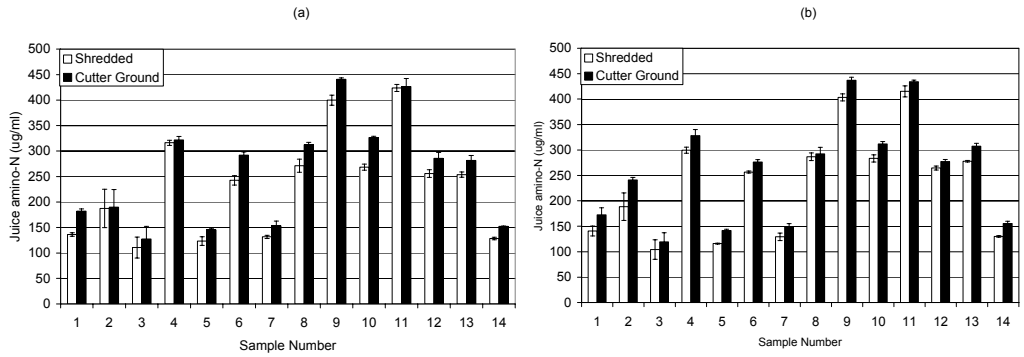


Figure 15: Extraction of amino-N from sugarcane fibre that has been shredded (□) or Jeffco cutter ground (■). Hydraulic press applied is either light (a) or heavy (b).

These findings are supported by the fact that the rind and nodal tissue of sugarcane has been found to have higher concentrations of amino-N than the pith tissue, a phenomenon exaggerated at higher amino-N concentrations (see Appendix 28). Rind and nodal tissue is harder than the pith tissue. The project team hypothesises that the Jeffco Cutter Grinder used to fibrate sugarcane fibre as part of the Jeffco/Carver extraction technique breaks up more of the rind and nodal cell tissue than the mill shredder does. When hydraulic pressure

is applied to extract juice in the Carver Press, more rind and nodal cells have been ruptured and the higher concentration of amino-N is extracted into the juice leading to an overall higher concentration of amino-N.

The level of cane preparation influences extraction of amino-N however the level of hydraulic pressure applied to cane fibre does not. The implication of this is the performance of the mill shredder will effect the extraction of amino-N into first expressed juice.

5.1.5 Investigation of laboratory determination of amino-N

An investigation into the performance of the laboratory technique used to determination amino-N levels in sugarcane juice is warranted to ensure this is not a source of variation that could reduce the ability of CAS NIR to predict amino-N.

5.1.5.1 Effect of juice filtering prior to ninhydrin assay

Sugarcane juice is filtered with a 0.8μm filter as part of the ninhydrin technique sample preparation process. There was some suggestion a filter this fine could bind proteins, and hence could possibly bind amino acids resulting in a lower amino-N being measured. Figure 16 compares the amino-N of samples where the 0.8μm filtration step is omitted from the sample preparation process.

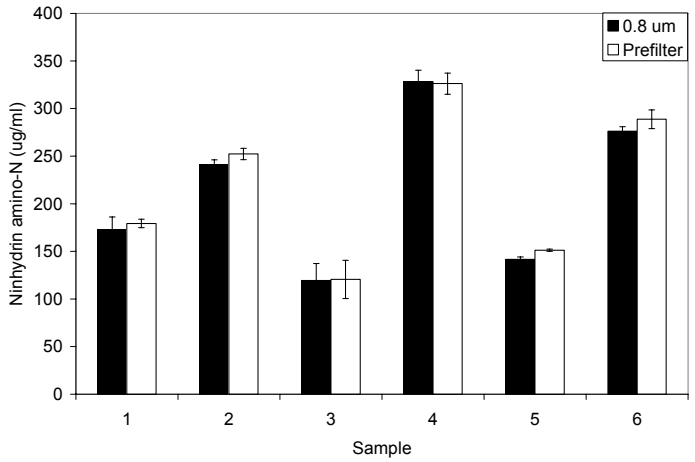


Figure 16: Amino-N of samples when either a prefilter (□) or a prefilter and 0.8μm filter (■) sample preparation is used prior to amino-N determination.

The filtering of juice with a 0.8μm filter prior to amino-N determination by the ninhydrin technique does not influence the amino-N.

5.1.5.2 Investigation of the ninhydrin assay

The ninhydrin assay determines the concentration of amino acids (nmol/ml) in sugarcane juice. An evaluation of the assay was conducted by performing HPLC analysis on 82 samples of Jeffco/Carver extracted juice from Mulgrave, 2001. HPLC determines the individual concentration of 18 amino acids (in

nmol/ml), which are summed to give an amino acid concentration of the juice. Figure 17 plots the relationship between the ninhydrin assay determination of amino acid concentration and HPLC determined amino acid concentration.

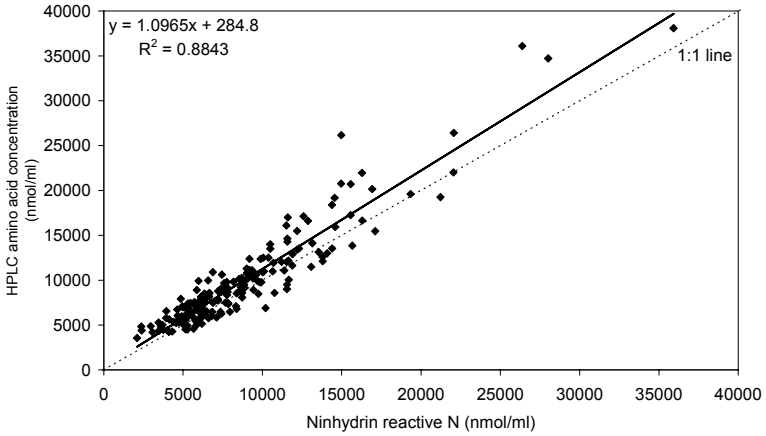


Figure 17: Comparison of amino acid concentration determined by ninhydrin assay and HPLC.

The ninhydrin technique is slightly underestimating the amino acid concentration as determined by HPLC, but the correlation is satisfactory. The ninhydrin assay is unlikely to be a source of variation in amino-N that adversely affects the ability of CAS NIR to calibrate for amino-N.

5.1.5.3. Investigation of the ninhydrin to amino-N conversion equation

Amino-N determined via HPLC (μg/ml) is compared with amino-N determined via ninhydrin (μg/ml) to evaluate the conversion equation used to convert amino acid concentration (as determined via ninhydrin) to amino-N. Figure 18 evaluates the performance of the conversion equation using 82 samples of Jeffco/Carver juice from Mulgrave 2001.

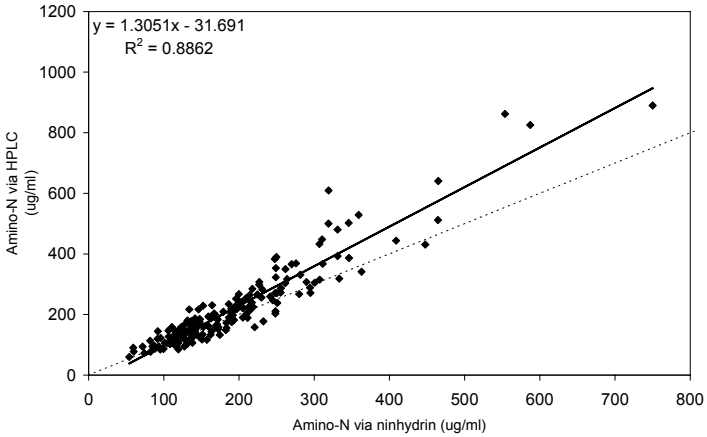


Figure 18: Evaluation of 1996 amino-N conversion equation.

Figure 18 shows ninhydrin amino-N value is slightly lower than HPLC amino-N when the 1996 conversion equation is applied to the data, particularly at high amino-N values. By plotting ninhydrin reactive N against HPLC derived amino-N an alternative conversion equation is estimated in Figure 19.

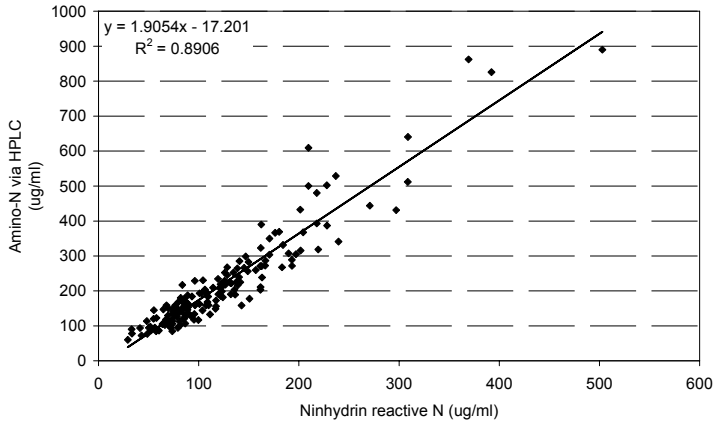


Figure 19: Relationship between ninhydrin reactive N and HPLC derived amino-N.

As this conversion is based on only 82 samples from a single mill area in a single year, and questions remain over the concentration of some amino acids, further investigation is required before the amino-N conversion equation is changed. Samples collected from 2002 are undergoing HPLC analysis to confirm this data.

The relationship shown here between ninhydrin reactive N and HPLC amino-N of y = 1.9054x – 17.201 is different to the 1996 conversion equation (y = 1.47x + 10.78). The newly developed equation is logical due to the high proportion of amino acids present in sugarcane juice that have two nitrogen atoms associated with them. Almost doubling ninhydrin reactive N (1.9054 x concentration of α amino acids) to determine amino-N makes sense when the number of 2N amino acids in cane juice is accounted for. Figure 20 plots a validation equation for ninhydrin determined amino-N when the new calibration equation is applied to the Jeffco/Carver data from Mulgrave 2001.

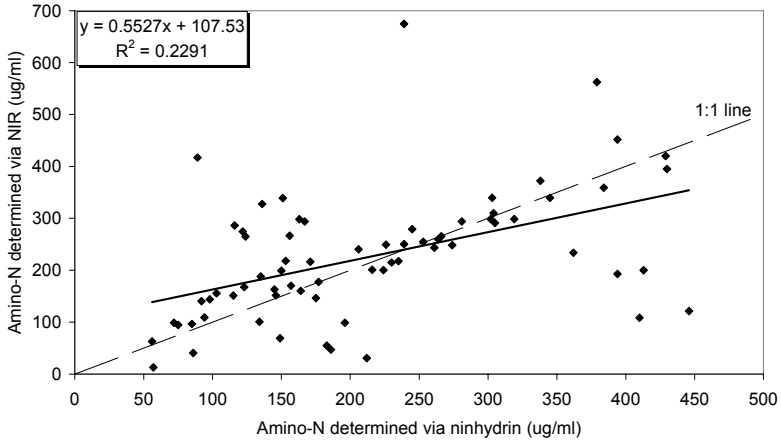


Figure 20: Validation plot of amino-N calibration developed from new calibration equation Jeffco/Carver extracted juice, Mulgrave 2001.

When amino-N is determined using this new conversion equation and an NIR calibration equation is attempted the validation of the equation is comparable with the validation plot for amino-N determined using the existing calibration equation (Figure 4).

The laboratory determination of amino-N does not appear to be contributing to the inability of CAS NIR to produce a robust calibration equation for amino-N. Slight improvements are possible from better understanding the ninhydrin technique, however these are not great enough to produce an improvement in the calibration equation.

5.2 Objective 2: Develop a communication plan identifying the key steps necessary for successfully implementing a nitrogen monitoring technique to an entire mill region

With a robust NIR calibration for amino-N developed it is possible to provide amino-N information to all growers in a mill region. The value a grower gets from this information depends on how amino-N data is applied on-farm. It is envisaged the amino-N of a block of cane would be used by growers, mill productivity staff and extension officers as part of the 'toolkit' used when managing nitrogen.

5.2.1 Communication plan

In lieu of this project choosing not to disseminate amino-N data to growers during the life of the project due to difficulties in obtaining a robust NIR calibration for amino-N a communication plan to achieve mill scale information dissemination was devised. Researchers can follow this communication plan once a robust calibration is developed. The communication plan was developed by following a framework for the development of extension processes (Foster 2000). The complete communication plan is provided in Appendix 15.

5.2.2 Reporting amino-N to growers

Through participatory practices with growers in the Mossman and Mulgrave mill regions an interpretation of amino-N data has been developed and two amino-N reporting mechanisms have been identified. One mechanism is to report amino-N data regularly throughout the course of the season and the other is to report on 'whole farm' performance at the conclusion of the season.

5.2.2.1 Amino-N interpretation

The amino-N value is in the units of μg amino-N/ml juice, and typically has a range between 80-700. The amino-N value does not relate to something tangible on farm. It was decided that amino-N presentation to growers would be more meaningful, and thus more likely to be utilised by growers, if presentation was in the form of ranges describing whether the amino-N is high, medium or low. These ranges are extracted from the work of project CTA029 that developed the diagnostic response curve shown in Figure 21.

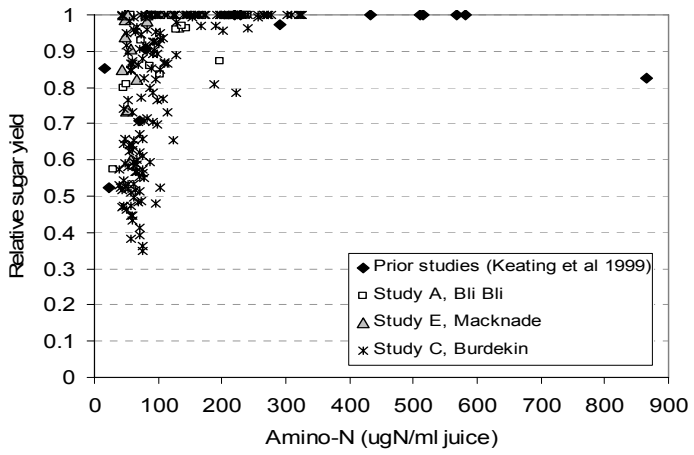


Figure 21: Sugar yield (as a fraction of yield in an N non-limiting treatment) versus amino-N in cane juice.

This diagnostic curve identifies amino-N values less than 150 μg amino-N/ml juice as being low, and those greater than 300 μg amino-N/ml juice as high. Table 6 summarises the proposed interpretation of the diagnostic relationship in Figure 21.

A document titled 'Guidelines for Cane Farmers on use of Amino Nitrogen Data' (Appendix 29) has been produced to interpret amino-N for farmers. The document was produced in consultation with growers and productivity staff from Mossman and Mulgrave mill areas. The guidelines describe amino-N as measuring the balance between crop demand for nitrogen and the nitrogen supply. This 'balance' description allows interpretation to include on farm factors that have an effect on amino-N such as those factors affecting crop yield and nitrogen supply. The guidelines are designed to accompany amino-N feedback to growers in the early stages of dissemination to help with interpretation.

Table 6: Summary of proposed interpretation of the diagnostic relationship between amino-N measured in cane juice at harvest time and relative sugar yield (where N supply is the factor limiting sugar yields).

Amino-N values (µg N/ml juice)*	**Description**	**Interpretation**
Less than 150	Low range	No suggestion of excess N supply. May indicate either optimal balance of N supply and demand or insufficient N resulting in sugar yield limitations. Monitor closely in subsequent years and consider N strip trials to test for N limitations.
150 to 225	Low Target range	No suggestion of either N excess or N deficits. Amino-N at the lower end of the target range.
225 to 300	High Target range	No suggestion of either N excess or N deficits. Amino-N at the higher end of the target range
Greater than 300	Excess range	Evidence of N excess, monitor in subsequent seasons and consider reasons for high N status (eg., excess N rates, recent mill mud additions, nitrate in irrigation water, fertile soil with high organic N status etc)

*Based on juice extracted after a 'Jeffco/Carver' extraction process.

5.5.2.2. In season amino-N reporting

All cane is sent to the mill as a rake unit, generally 10-100 tonnes of cane. Rakes are used by the mill to differentiate a homogonous unit of cane for payment purposes. A single block of cane may comprise one or many rakes, depending on the size of the block and timing of harvest. Because cane payment is based on the rake, growers receive regular in season reporting on details of all rakes they have sent to the mill. CAS NIR is designed to report on the rake unit, consequently amino-N data is available on each rake of cane sent to the mill.

During the season at regular, timely, intervals amino-N data should be provided to growers detailing basic rake data with the amino-N range for each rake. This feedback can ideally go with the regular mill rake feedback mechanisms, or be done as a separate feedback tool, possibly with other CAS NIR data not

detailed in rake reports. The timing of rake amino-N feedback, if not done concurrent with rake data, should be monthly or at a logical time such as the conclusion of each harvesting round.

An example of in season feedback rake reports, as designed in consultation with growers in Mossman and Mulgrave, is presented in Table 7.

Table 7: Sample of in season amino-N reporting on rake data.

Farm	Block	Sub-Block	Rake	Date	Variety	Class	Weight	CCS	Amino-N
####	1	1	43	3 Sept	Q124	4R	27.63	12.0	Low Target
####	1	1	44	3 Sept	Q124	4R	63.45	12.6	Low
####	1	1	283	3 Sept	Q124	4R	69.34	12.4	Low
####	7	0	285	5 Sept	Q138	Replant	56.93	13.3	Excess
####	7	0	882	5 Sept	Q138	Replant	68.63	14.0	High Target
####	2	1	908	5 Sept	Q107	Replant	64.32	14.1	Low Target
####	2	1	938	5 Sept	Q107	Replant	19.59	13.7	High Target

5.2.2.3 End-of-season amino-N reporting

At the conclusion of each season growers are provided with an end-of-season block performance report by the productivity department of the mill. This report summarises block productivity data from the season with cane yields and sugar yields presented on a block scale (block level data is summarised from rake data for each specific block). This reporting vehicle provides an opportunity to present summarised block amino-N data to all growers in a mill area.

Block amino-N values are calculated from each of the rakes that originated from a specific block. Weighted block amino-N values are presented as either low range, low target range, high target range or excess range as described in Table 6.

To aid in the presentation of block amino-N data thematic maps are produced. The thematic maps have amino-N low range, low target range, high target range and excess ranges colour coded for each block. A table summarising block amino-N descriptions accompanies these thematic maps. Table 8 and Figure 22 provide an example of end of season amino-N reporting.

Table 8: Sample of weighted block amino-N data in format for end of season reporting to accompany mill block productivity reports.

Farm	Block	Sub-Block	Variety	Class	CCS	Amino-N
####	1	1	Q124	4R	12.4	Low
####	7	0	Q138	Replant	13.7	High Target
####	2	1	Q107	Replant	14	Low Target

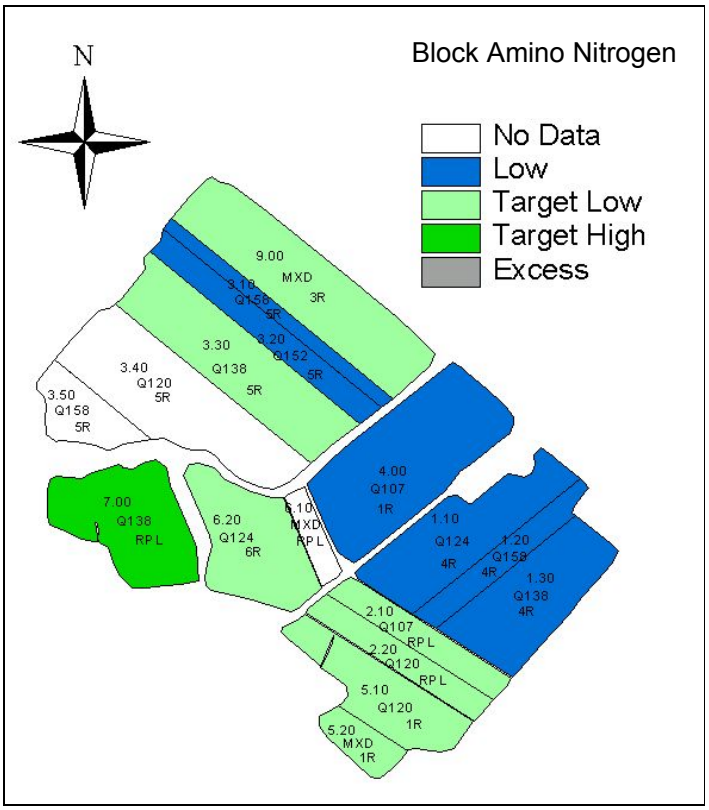


Figure 22: Sample of thematic map of weighted block amino-N data for end of season amino-N reporting.

5.3 Objective 3: Identify relationships between crop N status (as determined by laboratory based amino-N assays) and CCS at the block scale

Commercial cane sugar (CCS) is an estimate of the yield of cane sugar from sugarcane, (BSES 1994) i.e. how much sugar the mill can extract from sugarcane. There are many factors that influence the CCS of a rake of cane, these factors can be grouped into plant physiological factors and the growing/processing conditions the cane experiences. Evidence exists that excessive nitrogen uptake by the plant may contribute to a reduced sugar yield (Muchow *et al.* 1996). Amino-N assays of cane can be used to determine if excessive nitrogen potentially contributes to reduce CCS.

5.3.1 Factors that influence CCS

The many factors that contribute to the mill derived CCS of sugarcane can be grouped into plant physiological factors and the growing/processing conditions experienced by the cane.

Plant physiological factors influencing CCS may include the variety, crop class, age of crop at harvest and composition of the 'cane' that is processed. The growing environment experienced by sugarcane will influence the accumulation

of sugar, and consequently CCS. Growing conditions include weather (water, supply, radiation, temperature), soil (water and nutrient supply capacity), physical forces applied to the crop (wind, machinery damage), pathogen load (pests and diseases) and crop management (timing of operations, effectiveness of operations). Harvesting and transport conditions that may influence CCS include speed, condition of machinery, time of day and operator skill. Harvesting and transport conditions often differ temporally (within a day or within a year). The mill the cane is sent to and its factory efficiencies such as shredder operation may vary through time, potentially influencing CCS.

The factors that influence CCS may be intrinsically linked. For example a crop of a particular variety heavily supplied with nitrogen may be very 'trashy' and full of suckers. Consequently extraneous matter in the crop could be higher than average, which may affect the factories efficiency at extracting first expressed juice, where CCS is determined.

5.3.2 Data analysis – amino-N and CCS

There are complex interactions between plant physiology, the plants growing environment, harvesting, transport and mill operation that influence CCS. It is because of the many factors that contribute to the CCS of a crop that a large dataset is necessary when attempting to identify relationships between CCS and an external factor, such as amino-N. All potential variables within a population need to be represented in a relationship before the relationship can be described as valid.

Due to the inaccuracies of the CAS NIR is determining amino-N all analysis of relationships between CCS and amino-N are performed on samples that have laboratory determined amino-N.

5.3.2.1 Mill scale analysis

Figures 23, 24 and 25 show how drilling down through amino-N vs CCS data from 2000 is required before any crude relationship becomes apparent. Trend lines are added to indicate the general shape of the data.

Figure 23 shows no relationship between CCS and amino-N. Figure 24 removes the variability that may be inherent in the factory sugarcane is crushed at but still shows no relationship. Figure 25 'drills down' further to class level, where plant cane supplied to Mossman mill during 2000 may show a relationship. Figure 26 'drills down' further into the data to show relationships between CCS and amino-N from single varieties and/or ratoons cut at similar times.

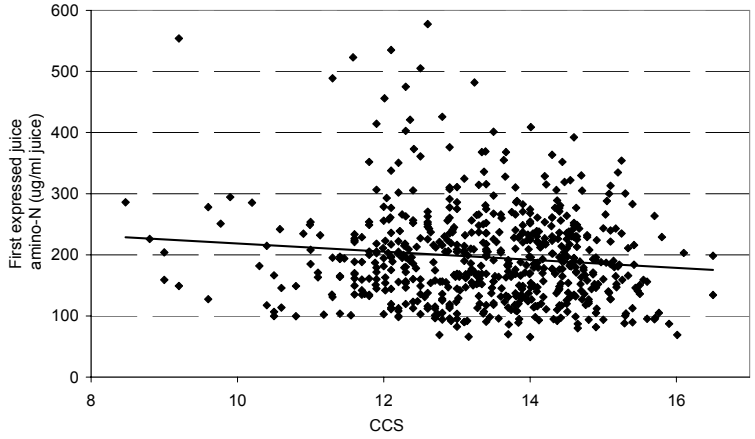


Figure 23: Relationship between amino-N and CCS from all samples analysed for amino-N during 2000 from Mossman and Mulgrave mills.

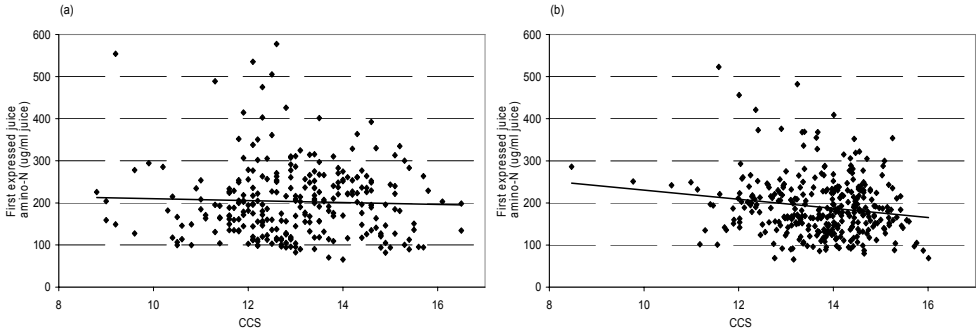


Figure 24: Relationship between amino-N and CCS from 2000 data from Mossman mill (a) and Mulgrave mill (b).

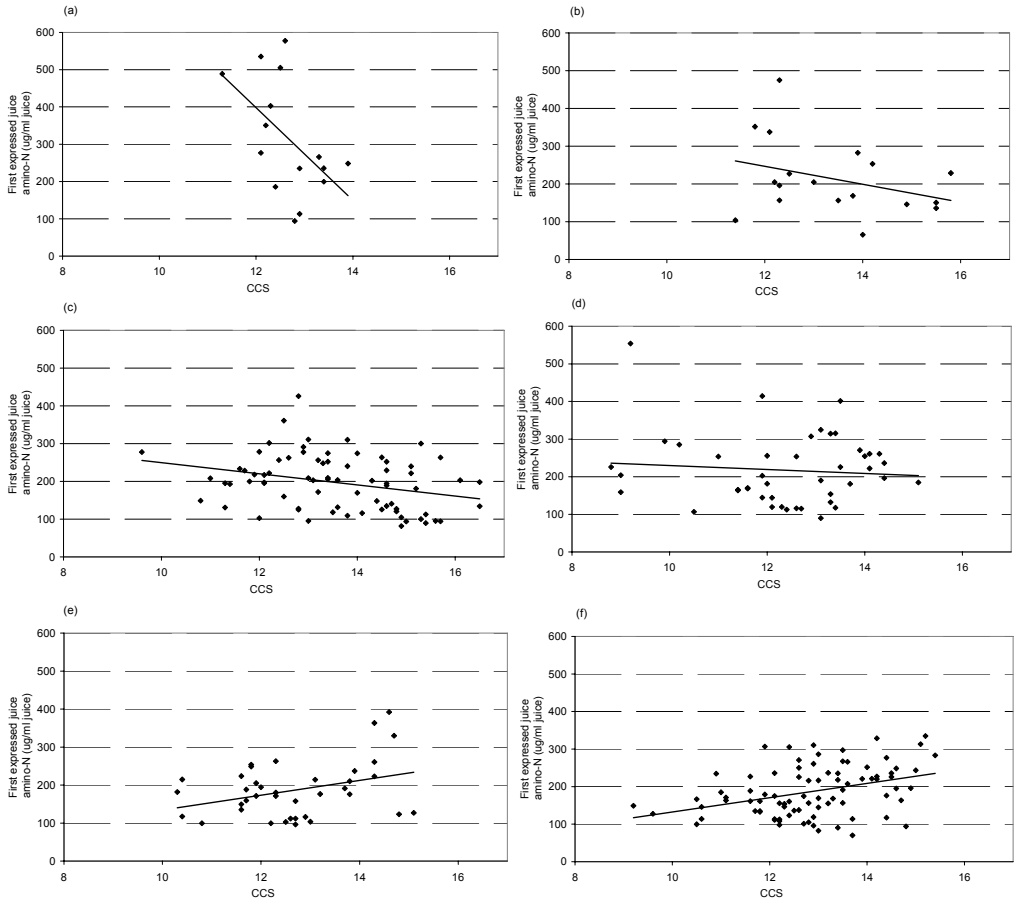


Figure 25: Relationship between amino-N and CCS from Mossman mill, 2000 for Plant cane (a), Replant cane (b), First ratoon (c), Second ratoon (d), Third ratoon (e) and Forth and older ratoon (f).

5.3.3 Summary of relationships between CCS and amino-N

There are many factors that may influence the final CCS measured in a mill's juice lab: physiological condition of the crop, the growing conditions of the crop, harvesting and transport and mill operation and efficiencies. This great source of variability in CCS means small data sets interpretations such as the one presented here need to be analysed with caution.

Drilling down through this limited data set has shown a tendency for high levels of amino-N to be associated with decreased CCS. While the figures presented support this, there are many occasions where this data set does not show this relationship, or any relationship. When amino-N is high, CCS tends to be low. This does not amount to the inverse of 'when amino-N is low, CCS is high'.

With a robust NIR calibration equation for amino-N operating at the mill the number of data points available for data interpretation increases by many orders of magnitude. Having the capacity to capture amino-N values from all rakes of cane in a season will allow potential relationships between amino-N and CCS to be better identified. In terms of how the amino-N information might be ultimately used to address CCS issue, Table 9 presents a logic argument

and Figure 27 provides a schematic of the relationship between CCS and amino-N that might ultimately emerge.

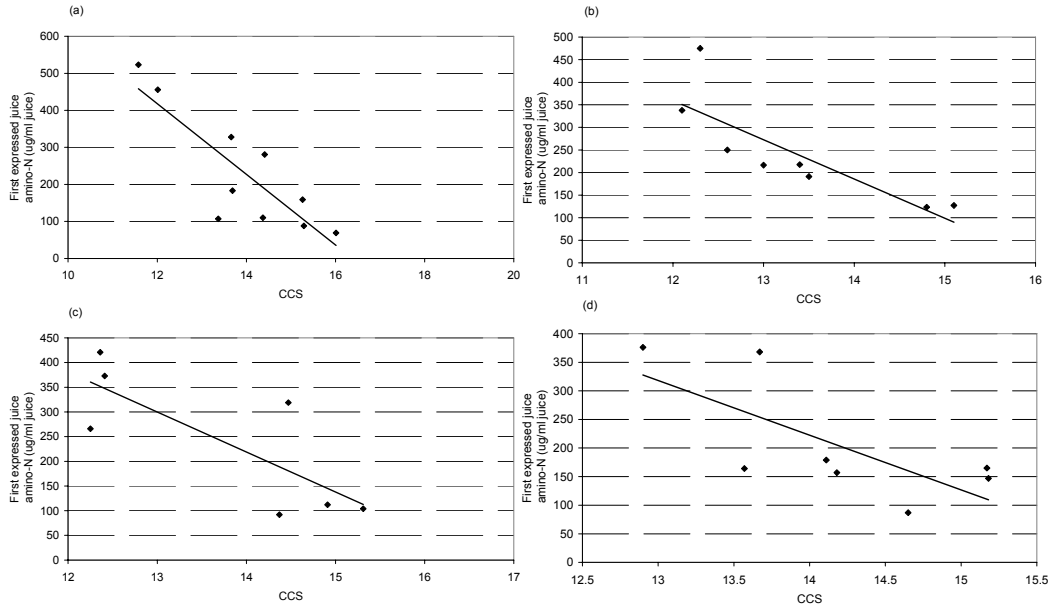


Figure 26: Relationship between CCS and first expressed juice amino-N from Mulgrave mill 2000. Q174, Plant and Replant (a). Q107 (b). Q96 (c). Q138, 3rd ratoon (d).

Table 9: How amino-N may be used to interpret CCS.

CCS	Amino-N	Interpretation
CCS low	Amino-N low	Nitrogen supply unlikely to be the cause of low CCS
CCS low	Amino-N high	Excess nitrogen supply may be contributing to low CCS
CCS high	Amino-N low	Low CCS not an issue
CCS high	Amino-N high	Low CCS not an issue

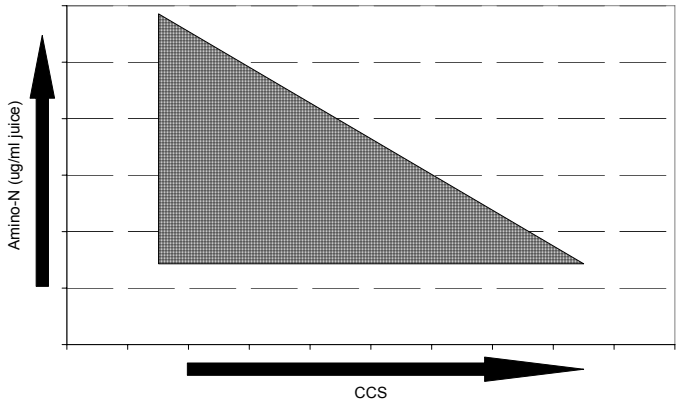


Figure 27: Schematic of a possible relationship between CCS and amino-N. Shaded area indicates the likely relationship space. Larger datasets are needed to adequately determine the nature of these relationships.

5.4 Objective 4: Explore the impact of the different components of cane supply (sound cane, suckers and extraneous matter) on the amino-N monitoring technique

The effect of adding various extraneous matter components to cane was investigated. Cane samples came from a plant crop grown with N rates varying from 0 to 180 kgN/ha. This plant crop was well supplied with N from soil reserves and only small responses in juice amino-N levels were observed (Figure 28). This lack of an N response is typical for a plant crop where there is often sufficient N in the soil to grow the crop.

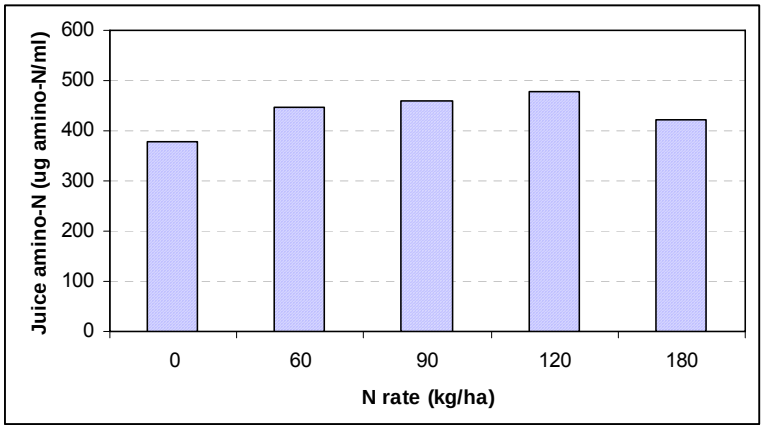


Figure 28: Juice amino-N results for end of season harvest of plant crop, September 1997.

Adding extraneous matter (stem, cabbage, leaf, soil or trash) had little effect on the amino-N levels measured by either HPLC (Figure 29) or ninhydrin assay (Figure 30).

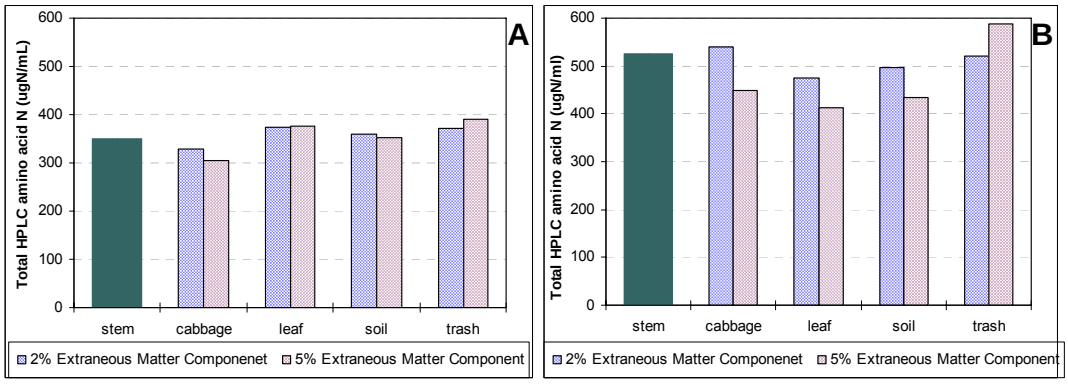


Figure 29: The concentration of amino acid N as determined by HPLC for each the EM additions. Two N rate treatments are 0 kg N ha^{-1} (A) and 180 kg N ha^{-1} (B).

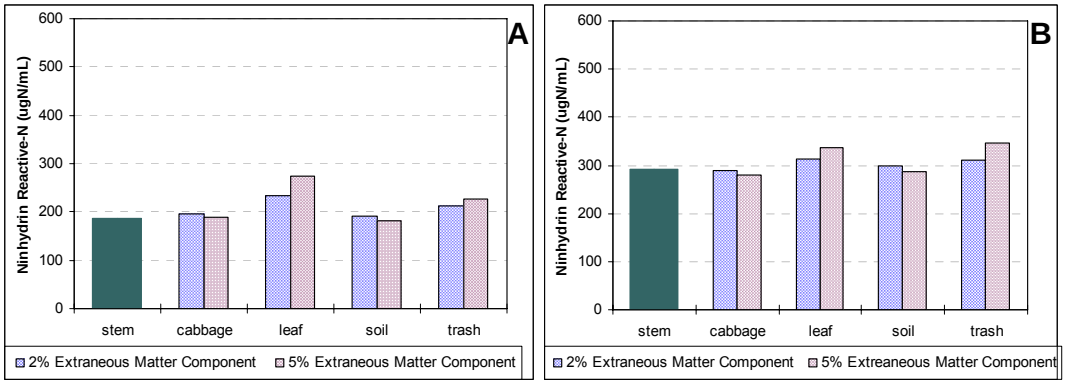


Figure 30: The concentration of amino N as determined by ninhydrin assay for each of the EM additions. Two N rate treatments are 0 kg N ha^{-1} (A) and 180 kg N ha^{-1} (B).

The lack of a change in the concentration of amino-N per ml extracted juice due to the addition of EM is not unexpected. The EM additions would be expected to add little amino-N to the sample and the slight increases in the concentration are probably artefacts of the extraction. The EM material could be absorbing some of the water in the extracted juice for example. This is highlighted in Figure 31, where the efficiency of juice extraction is illustrated. It can be seen that any addition of EM reduces the amount of juice extracted. The addition of trash EM causes the greatest reduction in juice extraction and the addition of as little as 5% trash resulted in 50% less juice extracted.

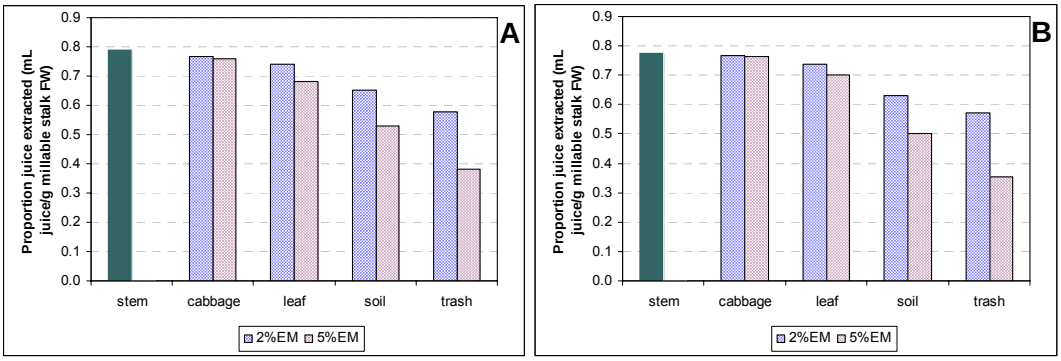


Figure 31: Juice extraction efficiency as affected by the addition of extraneous matter (EM) of different types. Stem has no EM added. Two N rate treatments are 0 kg N ha^{-1} (A) and 180 kg N ha^{-1} (B).

The results in Figure 31 are somewhat surprising. This data shows the addition of 50g of soil to 1000g of millable stalk results in a 36% reduction in the amount of juice extracted and the addition of 50g of cane trash resulted in reduction of 54%. Such high juice extraction reductions would have dramatic consequences in a mill where the level of EM is often higher than 5%. The degree of pressure used to extract juice was shown to not have an impact in an experiment to

investigate the effect of cane preparation and hydraulic pressure on extraction of amino-N.

The size of these extraction reductions seen in this experiment must be due to the interaction of the EM and the nature of the carver press method compared to the milling train of a commercial mill. This reduction in juice extraction results in increased concentration values for amino acids (Figure 32). This is especially evident with the 5% trash EM. The trash may be absorbing water from the juice hence concentrating the juice solutes.

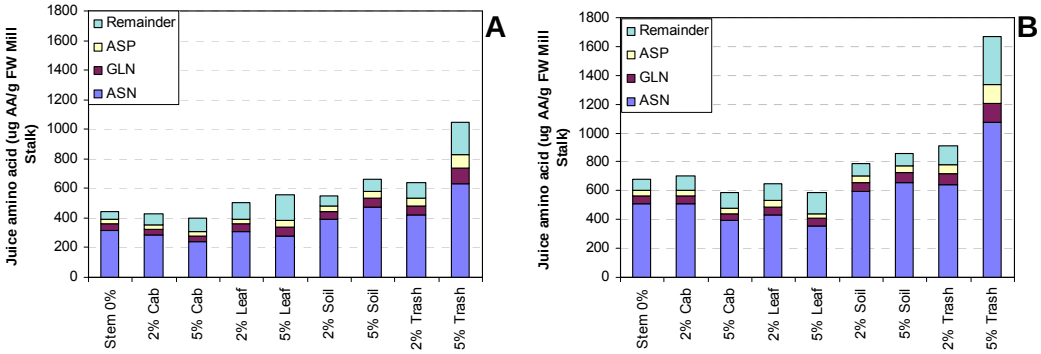


Figure 32: Amino acid concentration for juice extracted after the addition of extraneous matter from 2 N rate treatments (A = 0 kg N ha^{-1} and 180 kg N ha^{-1}).

Asparagine makes up just over 70% of the cane stem amino-N in the clean cane sample. This fraction rises to close to 80% in the cane receiving the 180kgN/ha fertiliser rate (Figure 33). These amino-N composition profiles were largely unaffected by the extraneous matter addition, except in the case of addition of large quantities of green leaf, something that increased the presence on of amino acids other than asparagine (Figure 33).

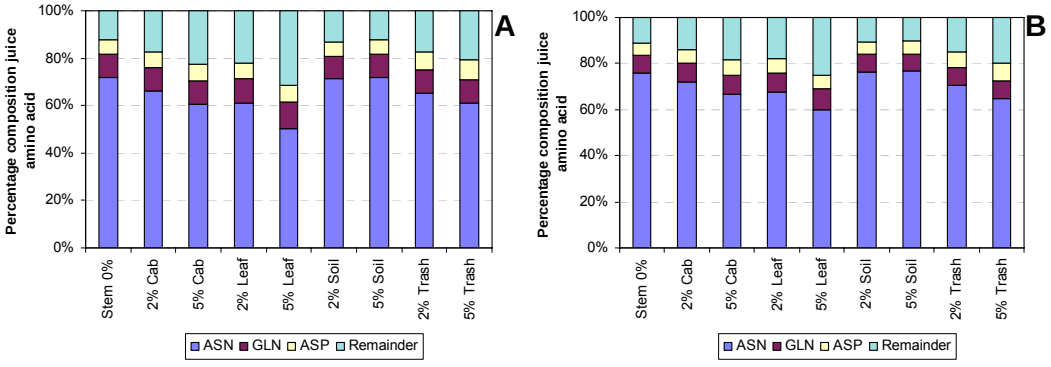


Figure 33: Relative amino acid composition for juice extracted after the addition of extraneous matter for 2 N rate treatments (A = 0 kg N ha-1 and 180 kg N ha^{-1}).

6. Outputs

The majority of project outputs relate to key understandings relevant to the objective of the development of mill-scale calibration of NIR as a practical tool in the routine monitoring of amino-N levels in cane supply. These outputs include;

- Transmission NIR was shown to be capable of measuring amino-N levels in first expressed juice, but only after the juice was filtered to remove the high levels of colloidal material generally present. On line filtering of first expressed juice was deemed problematical and inconsistent with industry moves in the direction of reflectance NIR of whole cane supply (i.e., CAS-NIR). Hence the project placed its focus on reflectance NIR approaches on whole cane supply.
- Three separate attempts confirmed our inability to calibrate NIR spectra recorded from whole cane supply, with amino-N levels measured in the laboratory on first expressed juice. The reasons for these calibration failures were investigated, in terms of robustness of the laboratory procedures in amino-N measurement and nature of the within mill processes that might influence the overall calibration precision.
- In terms of robustness of laboratory procedures, the project has shown;
    - Various pre-filtering techniques were not found to significantly influence the amino-N determination.
    - The ninhydrin assay was shown to slightly underestimate the amino-N concentration compared to an assay based on full determination and summation of individual amino-acids by HPLC methods. However, the correlations remain tight and the ninhydrin assay method was not considered to be a significant factor introducing variability into the CAS-NIR calibration process.
    - An improved conversion equation was sought relating ninhydrin determined amino-N to total amino N. A new conversion equation was proposed, but needs additional samples collected from 2002 analysed via HPLC to confirm its robustness. This equation is consistent with our basic understanding that indicates the amino-N in sugarcane stems is dominated by the amino-acid asparagine, which contains 2 atoms of nitrogen in every amino-acid molecule.
- In terms of nature of the within-mill processes that might influence the NIR calibration process, the project has shown;
    - The amino-N concentrations in first expressed juice is not fully representative of the total amino-N in the whole cane supply.
    - This “non-representativeness” relates more to the cane preparation technique (i.e. the level of fibration of the cane stem tissues and cells) than to the pressure applied in the of juice extraction process. Much better progress in NIR calibration development was possible when a combination of the Jeffco Cutter Grinder was used with a Carver Press to extract amino-N in a juice sample than proved possible with first expressed juice.
    - Methanol extracts of total amino-N in whole cane samples prepared by Jeffco Cutter Grinder confirmed that calibration was possible when a consistent extraction methodology was deployed.

- Significant enhancements in the calibration of CAS-NIR in estimating amino-N levels in cane supply were achieved via log transformations of the laboratory determined data to account for the non-normal distribution in the amino-N concentrations
- As an alternative to amino-N determination by CAS-NIR, an alternative calibration of total-N was demonstrated. This could prove useful in future work that aims to use total N removal in harvested product as an explicit source of information in guiding N fertiliser inputs.
- Project objectives that related to investigating the relationships between amino-N levels in cane stems and CCS levels were constrained by the methodological challenges encountered in the development of robust NIR calibrations. Nevertheless, limited investigations based on direct amino-N laboratory measurements have shown situations in which high amino-N levels have been associated with low CCS. Many factors influence CCS and cause and effect is difficult to ascertain. However, with the more extensive data capture possible with NIR based assessment, we would expect improved insights to emerge

    - Investigations of the effects of addition of extraneous matter to the determination of amino-N levels in cane stems revealed little effect on amino-N concentrations, but larger reductions in the quantities of juice that could be extracted. These physical effects of extraneous matter on juice extraction could have added to the difficulties encountered in our early attempts to calibrate NIR using first expressed juice. This provides further rationale to develop NIR calibration based on a direct measure of total amino-N in the whole cane sample, and not introduce the additional variable of the juice extraction process in the No 1 mill.

Additional outputs relate to strategies for communicating amino-N information to growers. These include;

- Through a participatory process with growers in Mossman and Mulgrave mill areas, two reporting mechanisms were developed and captured in a communications plan. This plan has the following features;
    - Within season reporting of amino-N via regular rake reports
    - End of season “whole farm” performance of amino-N monitoring via block specific maps.
    - A framework of reporting based upon four categories (low, target-low, target-high and excess), with specific guidance to growers associated with each category.
    - A document titled “‘Guidelines for Cane Farmers on use of Amino Nitrogen Data” produced in consultation with growers and productivity staff from Mossman and Mulgrave mill areas to assist in interpretation of “N at the Mill” monitoring.

7. Expected outcomes

This project has been directed at providing a technique that would enable sugar growers to universally monitor the N status of their crops and adjust N fertiliser rates accordingly. The potential outcomes of such a technique, should it be universally adopted, include;

- Significant reductions in the costs of nitrogen fertiliser inputs through better targeting of fertiliser rates and a reduction in rates in situations were evidence of excess N supply is uncovered. Conservatively, a 20% reduction in N rates on 50% of farms. This would represent and annual saving in the order of $7M p.a.
- Reductions in N losses to the environment, conservatively estimated at half the reductions in N fertiliser inputs.
- Enhanced image for the Australian sugar industry with the Australian and international community. With high levels of government and community concern over loss of nutrients to waterways feeding into the Great Barrier Reef lagoon, the significance of such a highly visible and potentially widespread effort to improve the efficiency of N fertiliser management cannot be underestimated.

The project has not yet achieved these outcomes, as it encountered significant technical obstacles to widespread application. These challenges now appear to have been largely overcome as the project comes to a close, but the need for the approach to improving N fertiliser management effectiveness remains.

8. Future research needs

The project team had wanted to make further progress in this project than proved ultimately possible. We had hoped to deploy the “N at the Mill” approach in one or more mill areas and use a participatory / action research approach to shaping how growers would use the new information and to assessing its value. Technical obstacles in the NIR calibration process substantially reduced what we could achieve in the project timeframe. These obstacles are now largely overcome. The only note of caution we would add is we still have more to learn about the robustness of the amino-N / CAS-NIR calibration across years and mill regions. The validations developed to date use samples and spectra that are not the same as those used in building the calibration (i.e., the redundant spectra), but these data come from the same years and mill regions as did the calibration spectra. Hence, it would be wise to continue to test the robustness of NIR calibrations, in a way informed by the results of this project.

Assuming that the calibrations do hold up to further independent validation, the original plans to deploy this technique in a mill region and work in case study mode with growers in its application remain valid.

9. Recommendations

We recommend;

- That independent validation of the CAS-NIR calibrations take place in the 2003/04 crushing season
- A new project be established in 2004, that deploys "N at the Mill" monitoring at a whole mill region scale and deploys participative research capability to work with selected grower groups in interpretation of the new N monitoring information and in exploring appropriate grower responses. Such a project may be attractive to industry groups involved in the current industry restructuring to meet economic and environmental imperatives.

10. Acknowledgements

Many individuals and organisations have made important contributions to this project. Most significant of these has been Steve Staunton of the BSES in Meringa. Steve has provided NIR expertise to the project since its inception and his constructive input is gratefully acknowledged. Sincere thanks also goes to growers and mill staff in Mossman, Mulgrave, South Johnstone and Tully who have assisted in various aspects of the project's operations. Particular mention must go to Chris Hoare and Alan Rudd from Mossman, Glen Pope and Trevor Crook from Mulgrave and Rob Stobbie from Tully mill.

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Appendices (separate volume)

Sugar Research and Development Corporation

PROJECT CTA045

APPENDICES TO:

IMPROVING CCS IN THE WET TROPICS VIA BLOCK-SPECIFIC MONITORING OF N IN CANE DELIVERED TO THE MILL

B.A. Keating, A.J. Webster, and I.M. Biggs

CSIRO Sustainable Ecosystems
Level 3 Queensland Bioscience Precinct
306 Carmody Road
St Lucia 4067

FINAL REPORT

June 2003

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Appendix 1: Duty statement for the position of project officer, awarded to Mr Tony Webster.

Research Agronomist

Action research in the sugar industry

CSIRO Tropical Agriculture, Cairns Qld

$44- $57k + Superannuation

3 year term appointment

CSIRO Tropical Agriculture conducts leading edge research on the management of agricultural systems in the areas of profitability, resource sustainability and environmental protection. Our research on better management of nitrogen in sugar systems has identified an opportunity to use the monitoring of nitrogenous compounds as cane is processed at the mill, to guide N management practices on the farm. This work is supported by the Sugar Research and Development Corporation and links strongly with that of a BSES-Sugar North consortium, that is developing applications of NIR technology in sugar milling operations.

We now wish to appoint an agronomist to take on responsibility for the day to day management of these research activities in the wet tropics. The appointee will work closely with growers, advisors, and milling personal in the region and link with the research team located elsewhere in the state. An action learning approach is planned, with growers, advisors and milling staff closely involved in research and delivery activities. The successful applicant will have a post-graduate degree or equivalent professional experience in an agricultural science related discipline. Good data analysis and communication skills are essential. Experience in participative or action learning approaches to research would be an advantage.

For a copy of the duty statement and the selection criteria please contact Cathy Simpson on phone number 07-32142318, fax 07-32142420, or email at Cathy.Simpson@tag.csiro.au

Written applications **must** address the **selection criteria** and include the names and contact details (including phone, fax numbers) of at least two professional referees. All applications should be marked ‘Confidential’, quoting the appropriate reference number and be forwarded to the Recruitment Officer, CSIRO Tropical Agriculture.

CSIRO is an Equal Opportunity Employer

Duty statement

The agronomist will work under the direction of the Group Leader and other senior research scientists in the Sustainable Sugarcane Systems Group of CSIRO Tropical Agriculture. In the first instance, the appointee will be based in Cairns, in the offices of Sugar North Limited. He/she will work with growers, grower groups, and mills throughout the wet tropics and liase closely with other team members located in CSIRO Townsville and Brisbane.

The officer will be responsible for the implementation of the project titled, "Improving CCS in the wet tropics via block-specific monitoring of N in cane delivered to the mill ". This project, with the support of the Sugar Research and Development Corporation, is developing a technique of assessment of the N status of cane crops by monitoring the levels of amino nitrogen in the cane as it is processed at the mill. In this case, the focus is on establishing the value of this technique to canegrowers and millers in the wet tropics. This work is being done in the context of declining levels of CCS in cane supply and uncertainty on the role of nitrogen management in this decliine.

The officer will be responsible for planning and implementing a work program that delivers on milestones defined in the project agreement. This will involve ;

- Supervision of experimental activities involving collection and processing of plant and soil samples for analyses conducted by other members of the project team.
- Monitoring of on-farm experiments jointly planned and implemented with growers.
- Participative research activities with canegrowers, productivity groups, industry advisors and milling staff that are consistent with the action learning cycle of planning, action, reflection and further planning.
- Benchmarking activities that document current practices and knowledge (with respect to sustainable N management) and identify the benefits accruing from project activities.
- Research on effective means of communicating the outputs of project research to growers and advisors.
- Higher-order data analysis, interpretation and presentation suited to direct transfer into project reports.
- Liaison with other researchers whose skills are needed to deliver on project objectives (e.g. spatial information systems researchers from the Sugar CRC, NIR researchers from BSES/Sugar North)
- Communication with key industry clients to promote improved understanding of industry requirements and project achievements
- Preparation of project reports, research papers and industry communications.

CSIRO

Terms for Collaborative Research Agreement

Appendix 2: Research Agreement between BSES, Sugar North and CSIRO Tropical Agriculture, relating to development of amino-N calibrations for NIR.

COLLABORATIVE RESEARCH AGREEMENT

This is an Agreement between CSIRO and the Collaborator to carry out collaborative research in accordance with the following Details.

Details	
CSIRO	Commonwealth Scientific and Industrial Research Organisation, of Limestone Avenue, Campbell in the Australian Capital Territory, through the Division of Tropical Agriculture
Division	Name Tropical Agriculture Address 306 Carmody Rd St Lucia, 4067 Contact Person .Dr Brian Keating Telephone 07 32142373 Fax 07 32142420 e-mail brian.keating@tag.csiro.au
Collaborators	1. Collaborator name Sugar North Ltd. Collaborator address PO Box 467N Cairns North 4870 Collaborator contact Dr Scott Grimley : General Manager Collaborator telephone .07 40317088 Collaborator fax 07 40316916 Collaborator e-mail sugarnorth@iig.com.au 2. Collaborator name BSES Collaborator address PO Box 651 Bundaberg Q 4670 Collaborator contact Mr Ross Ridge : Senior Agronomist Collaborator telephone 07 41593228 Collaborator fax 0741593383 Collaborator e-mail
Project	**Collaboration on monitoring N composition of cane supply at the mill.**
Purpose	• **To demonstrate the potential to use the SugarNorth/BSES system for Near Infrared Spectroscopy (NIR) based monitoring of the prepared cane stream in sugar mills to assess the levels of amino-N in cane supply to sugar mills.**

Start Date	*20th September 1998*
CSIRO Background IPR	*CSIRO's background intellectual property is held jointly with the partners in the SRDC supported projects, CSC21s (now complete) and CTA029 (ongoing). These partners, and their respective equity in intellectual property for CSC21s outputs are : CSIRO (49.3%), BSES (8.8%), CSR (8.8%) and SRDC (33.1%). The equity arrangements in the current CTA029 project are : : CSIRO (35%), BSES (10%), CSR (10%), Bundaberg Sugar (10%) and SRDC (35%).* This background intellectual property is all related to a system for relating N composition of sugarcane stems at harvest time (e.g., at the milling step) to the N supply conditions that cane experienced during its growth and development. This information on prior crop N status is intended to be used in the N management of subsequent crops from the specific farm and block. Specifically the system is made up of ; - Knowledge of the nitrogen composition of cane stems and the partitioning of different N forms between juice and non-juice fractions. - Relationships between amino-N (and other N forms) in juice and whole cane stalks and crop performance (growth, sugar yield etc) in the field under defined soil and management conditions. - Information on the variability in amino-N composition in cane supply in Herbert River and Bundaberg mills. - Relationships between full HPLC analysis of amino-N composition of cane stems and juice and more rapid means such as the “Ninhydrin-reactive method”. - Preliminary analyses that indicate that amino-N levels in cane juice can be calibrated to features of Near Infrared Spectroscopy (NIR) - Information on the effect of various forms and levels of extraneous matter on the levels of amino-N in cane juice.
Collaborator Background IPR	Sugar North Ltd and BSES have been collaborating in an R&D Joint Venture to develop an on-line direct cane analysis system based on Near Infra-Red Spectroscopy (NIR). Whilst this project is continuing, the current conclusion is that many variables can be accurately predicted from the NIR spectra collected on each rake and related processing of those spectra. Specifically, the background intellectual property consists of ; - A “front-end” to an NIR system capable of presenting bulky, heterogeneous materials such as prepared cane supply with acceptable results in terms of calibration precision and predictive ability. - Demonstration (via the development of calibration equations and conduct of validation tests) of the value of this system for a wide cane of measurements on cane composition. The most relevant measure to this collaborative research proposal is the

	measurement of total N in whole cane stalks via laboratory analysis and its prediction via the NIR method.
Materials	**Supplied by Sugar North / BSES Joint Venture** - Samples of prepared cane and juice collected at a frequency and location as determined by Sugar North/BSES team - Samples of prepared cane and/or juice collected by the CSIRO team, as from time to time agreed between the parties. - Spectral information will be collected by SugarNorth/BSES on the rakes of prepared cane from which the samples referred to above were collected. - Prediction equations for estimating N status of cane supply from NIR spectra will be developed by SugarNorth / BSES based on the matching of the spectral data (gathered by SugarNorth/BSES) and the laboratory data (supplied by CSIRO team). These equations will be loaded onto installed NIR cane analysis systems and the outputs made available to the CSIRO team under conditions of confidentiality. **Supplied by CSIRO team** - *Analytical results on amino-N composition of cane samples as supplied. These data will consist of a mix of both full amino-N analysis via HPLC and more rapid determination via the "Ninhydrin Method". The number of samples analysed and nature of the analysis (e.g. prepared cane or juice analyses, ninhydrin or HPLC analyses) will be at the discretion of the CSIRO team, acknowledging both the need for as large a dataset as possible and the need for a realistic laboratory workload.* - *Research results on the amino-N monitoring system for all cane producing areas in Australia where such results are available. Specifically, these will be provided via publications, presentations to sugar industry forums, milestone reports and final reports to SRDC.*
Governing Law	State of Queensland

CSIRO

Terms for Collaborative Research Agreement

Date:

This Agreement to collaborate is made up of the Details, the Project Plan and Terms

.....	.....
Signed for Sugar North Ltd	*Signed for CSIRO*
.....	.....
Name and title	*Name and title*
.....	.....
Witness	*Witness*
.....	.....
Name	*Name*

.:.....

Signed for the BSES

.....

Name and title

.....

Witness

.....

Name

Project Plan	
Background	Researchers from the Division, Bureau of Sugar Experiment Stations (BSES), CSR and Bundaberg Sugar have been working on ways in which sugarcane growers can improve the precision of their nitrogen fertilisation. This work is aimed at reducing fertiliser costs to growers and reducing the risk that excess nitrogen fertilisers escape into ground or surface waters. The work is supported by the Sugar Research and Development Corporation (SRDC). A project, know as **"Monitoring N at the Mill"** has shown that the levels of N in cane stems as sampled at the mill can provide a guide to the N status of the crop. Specifically, the work measures the level on N containing amino-acids in the cane supply (referred to as amino-N) and relates this to know levels of adequacy or excess. One of the objectives of the current SRDC project (CTA029) is to identify rapid and cost effective means of amino-N analysis potentially suited to a milling setting. Preliminary analysis indicates NIR may have some potential for these analyses. A connection is being established between this work and that of the CRC for Sustainable Sugar Production. This connection specifically deals with the use of information on cane N status in the context of spatial information systems, linking field and factory data. The contact for the CRC linkage is Mr Stephen Routley of James Cook University (JCU). Sugar North Ltd (SNL) and BSES have been collaborating in an R&D Joint Venture project to develop an on-line direct cane analysis system based on Near-Infra Red Spectroscopy (NIR). Whilst this project is continuing, the conclusions able to be reached are that many variables can be accurately predicted from the NIR spectra collected on each rake of cane and the related processing of these spectra. The parties wish to collaborate in the development of robust global equations for amino-N in cane supply using NIR spectra. The results would be utilized by both the Division and Sugar North / BSES joint venture to enhance the sustainability of the Australian sugar industry.
Objectives	To demonstrate the potential to use the SugarNorth/BSES system for Near Infrared Spectroscopy (NIR) based monitoring of the prepared cane stream in sugar mills to assess the levels of amino-N in this cane supply. To explore the value of this information on amino-N levels in cane supply to cane growers management of nitrogen at the farm and block scale.
Tasks	**SugarNorth / BSES tasks**

	1. Collect samples of prepared cane, juice and collect associated NIR spectral data. The samples will be stored and preserved in a manner agreed between the parties for subsequent analyses. 2. Develop prediction equations based upon the stored spectra and analytical results and provide the CSIRO team with details of the performance of these prediction equations and their likely accuracy (eg. standard error of prediction, correlation co-efficient, slope and bias). 3. Make data available to the CSIRO team relevant block data (yield, CCS, variety, crop class etc) that can aid interpretation of the cane amino-N analyses. Provision of block data is conditional on the consent of the relevant mill and confidentiality of individual grower information would be maintained.. **CSIRO's tasks** 1. Undertake laboratory analysis for amino-N on cane juice and whole cane stem samples using a mix of methods deemed appropriate to the circumstances. 2. Provide data on amino-N composition of cane juice and/or whole cane stems to the Sugar North/BSES team for construction of NIR calibration equations. These data will include a measure of the uncertainty (or errors) associated with the laboratory analysis procedure.		
Milestones	Milestone	Milestone Target Date	Milestone Payment
	SugarNorth/BSES to collect juice and prepared cane samples progressively throughout the 1998 crushing season	December 1998	$nil
	CSIRO to collect additional samples as determined from time to time between the parties	December 1998	$nil
	SugarNorth/BSES to provide CSIRO with whole cane and juice samples	March 1999	$ nil
	CSIRO team to provide SugarNorth/BSES with results of laboratory analyses of amino-N	May 1999	$ nil
	SugarNorth / BSES to develop NIR calibration equations (and make details available on a confidential basis to CSIRO team)	June 1999	$nil
	CSIRO and SugarNorth / BSES to use an independent set of	July 1999	$ nil

	laboratory analyses to evaluate the performance of the predictive equations.		
	Total Fees		$ nil
Deliverables	• Predictive equations relating NIR spectra to amino-N composition of cane supply.		
Project Meetings	As deemed necessary by the consensus agreement of the Project Leaders We envisage a meeting would be held in June 1999 to review the results of collaborative work to date and determine whether additional collaboration was warranted in the 1999 crushing season.		

Project Leaders				
		CSIRO - Division	SugarNorth / BSES JV	BSES
	Name	Brian Keating	Scott Grimley	Ross Ridge
	Title	Senior Principal Research Scientist	General Manager	Senior Agronomist
	Contact Details	306 Carmody Rd St Lucia, 4067 Ph : 07 32142373 Fax: 07 32142420 e-mail : brian.keating @tag.csiro.au	PO Box 467N Cairns North 4870 Ph :07 40317088 Fax : 07 40316916 e-mail :sugarnorth @iig.com.au	PO Box 651 Bundaberg Q 4670 Ph 07

MEANING OF WORDS

In this Agreement:

'Background IPR' means any IPR created independently of the Project which a party makes available to carry out the Project and includes at the date of this Agreement the Background IPR of each party listed in the Details.

'Confidential Information' of a party:

means any information that a party claims is confidential to itself and marks as confidential at the time of disclosure or, where this is not possible, confirms in writing as being confidential within 14 days after disclosure;

in the case of CSIRO includes the information in CSIRO's Materials;

in the case of the Collaborator includes information in the Collaborator's Materials;

does not include information to the extent that information is:

independently developed or known by the other party (including because it is in the public domain); or
required to be disclosed or retained by law.

'Details' means the details to which these terms are attached.

'Intellectual Property Rights or IPR' means all intellectual property rights, including:

(a) patents, plant breeder's right, copyright, rights in circuit layouts, registered designs, trade marks, and any right to have confidential information kept confidential; and

(b) any application or right to apply for registration of any of the rights referred to in **(a)**.

'Management Committee' means a management committee established under **clause 2** (Management Committee)

'Milestone Report' means a report detailing progress of the Project for each Milestone.

'Personnel' of a party means that party's employees, agents and contractors.

'Project IPR' means all IPR developed under this Agreement other than the Deliverables.

.

'Project Results' means the Deliverables and the Project IPR.

'Project Plan' means the attached project plan.

'Report' means a Milestone Report or Final Report as described in **clause 6** (Reports).

Other words starting with a capital letter are described in the Details or the Project Plan.

MANAGEMENT COMMITTEE

The parties will set up a Management Committee to be responsible for the overall relationship between the parties and to oversee the Project.

Each party:

must appoint two representatives ('Research Coordinators'**) acceptable to the other party as its representatives on the Management Committee;**

may replace a representative by giving reasonable prior notice to the other party; and

may appoint an alternate person to act as its representative at a meeting but only if it notifies the other party at least 24 hours before the relevant meeting.

The Research Coordinators will arrange meetings of the Management Committee at each Milestone or more frequently if required to:

oversee the conduct of the Project;

consider Milestone Reports; and

if requested by the parties, discuss and recommend to the parties any proposed variations to any aspect of the Project.

Each party must ensure that for each meeting (for example, with each party doing the following for alternate meetings):

where possible, an agenda is circulated in advance; and

minutes are kept and promptly circulated to each party.

A quorum for meetings of the Management Committee is at least one representative of each party.

BACKGROUND IPR AND MATERIALS

Each Party

retains ownership of its Background IPR and Materials;

grants the other party a non exclusive, non transferable, royalty free licence of its Background IPR and Materials to the extent necessary to enable the other party to carry out its obligations under this Agreement; and

may only use any Background IPR and Materials of the other party outside the Project if it obtains prior written consent from that party.

The Collaborator:

bears all risk of loss or damage to its Materials while in transit or at CSIRO; and

acknowledges that its Materials may be altered, damaged or destroyed during conduct of the Project.

CSIRO may store, dispose of or destroy the Collaborator's Materials if:

there are no Return Instructions in the Details; or

the Client does not promptly make arrangements for their return in accordance with the Return Instructions.

WORKING TOGETHER

Each party must:

co-operate fully with the other party and any other person involved in the Project;

attend Project meetings as set out in the Project Plan;

ensure that its Personnel carry out the Tasks allotted to it:

in accordance with the Project Plan;
with due professional care and skill; and
under the supervision of the Project Leaders; and

supply the other party promptly with information the other party reasonably requires so that it is not delayed in performing its obligations under this Agreement.

Each party acknowledges that the other party may carry out research and development independently of the Project.

REVISIONS

*Either party may request revisions (including deletions or additions) to the Project by giving a notice to the other party (**'Request'**) specifying at least:*

the proposed revisions to the Project; and

any resulting changes to the Details or Project Plan, including any proposed change to the Total Fees.

Within 14 days after receiving a Request, the receiving party must notify the requesting party that it either:

accepts the Request, in which case the parties will sign and date it, which will alter and form part of this Agreement;

wishes to negotiate the Request; or

rejects the Request.

Until a Request is signed and dated by the parties:

that Request is not binding on the parties; and

the parties must continue working in accordance with the unaltered Agreement..

REPORTS

*CSIRO must submit to the Management Committee at each Milestone a **'Milestone Report**' specifying:*

the tasks performed for the Milestone;

any Project IPR generated; and

any proposed variations to the Project Plan.

*Within 30 days after the final Milestone, or, in the case of early termination, within 30 days after termination, CSIRO must submit a '**Final Report**' to the Management Committee specifying all tasks performed during the Project and all Project IPR generated.*

The Collaborator must provide all information requested by CSIRO to enable CSIRO to prepare the Reports.

PROJECT RESULTS

CSIRO:

assigns ownership of Deliverables to the Collaborator;

owns the Project IPR;

(c) grants the Collaborator a non exclusive royalty free right to use the Project IPR and Background IPR to the extent necessary to use the Deliverables

If the Collaborator wishes to obtain further rights to use the Project IPR:

it must send CSIRO an 'Exercise Notice**' within 60 days after it receives the Final Report;**

the Collaborator and CSIRO must within 30 days after the date of the Exercise Notice agree on the 'Details' of the attached Licence Agreement and sign that Licence Agreement; and

if the Collaborator and CSIRO acting reasonably are unable to finalise the Licence Agreement within the specified period CSIRO may, but need not, terminate negotiations and the option will lapse.

CONFIDENTIAL INFORMATION

*Each party (**'Recipient'**) must in relation to the Confidential Information of the other party (**'Discloser'**):*

keep it confidential;

use it only as permitted under this Agreement;

only disclose it to an 'Authorised Person';

not copy it or any part of it that is in material form other than as strictly necessary and must mark any such copy 'Confidential - (Discloser)';

promptly comply with any request by the Discloser to return or destroy any or all copies of Confidential Information.

An 'Authorised Person' means

any of the Recipient's employees:

who have a need to know (and only to the extent that each such employee has a need to know); and
who have first been directed and have undertaken orally or in writing to keep it confidential and to use it only as permitted under this Agreement ('Undertaking');

other people such as contractors, agents and visitors:

who have a need to know (and only to the extent that each such person has a need to know); and
who have agreed in writing to keep it confidential in accordance with this Agreement (also an 'Undertaking');

The Recipient must:

implement security practices against unauthorised copying, use and disclosure(whether that disclosure is oral, in writing or in any other form);

enforce each Undertaking; and

immediately notify the Discloser if the Recipient becomes aware of any:

unauthorised copying, use or disclosure in any form; or
disclosure required by law.

The burden of showing that any Confidential Information is not subject to the terms of this Agreement will rest on the Recipient.

WARRANTIES AND EXCLUSIONS

CSIRO does not warrant that the Project Results will not infringe any third party's IPR but will advise Collaborator if it becomes aware of any infringement.

*The Trade Practices Act 1974 and corresponding legislation in other jurisdictions in certain circumstances imply mandatory conditions and warranties into contracts ('**Consumer Warranties'**). This clause does not exclude or limit the application of any Consumer Warranties or other warranties where to do so:*

would contravene the law of the relevant jurisdiction; or

cause any part of this clause to be void.

Each party excludes:

all terms, conditions and warranties implied by custom, the general law or statute except any Consumer Warranties; and

all liability to the other party for consequential damage (including but not limited to, lost revenue or lost profit or loss of data) suffered by the other party in any way relating to the other party's use of the Project Results.

A party's liability to the other party for breach of any Consumer Warranty is limited, at the first party's option, to:

for services:

providing those services again; or
paying the cost of having those services provided again;

for goods:

replacing the goods that breach the warranty;
paying the cost of replacing the goods that breach the warranty.

LIABILITY

The Collaborator:

acknowledges that:

the Project Results are only provided for the Purpose; and
it exercises its rights to use and disclose the Project Results under this Agreement at its own risk; and

except to the extent caused directly by CSIRO's negligence or breach of this Agreement, assumes all risk for any liabilities, expenses, losses, damages and costs (including legal costs on a full indemnity basis and whether incurred by

or awarded against a party) incurred by CSIRO and resulting directly or indirectly from the Collaborator's use or disclosure of the Project Results under this Agreement.

DISPUTE RESOLUTION

The parties intend to resolve disputes without starting arbitration or court proceedings. Accordingly, each party will negotiate in good faith to resolve any dispute that arises out of his Contract and will involve in those negotiations a senior officer who has not been involved with the Research.

TERM OF AGREEMENT

This Agreement begins on the Start Date and, unless a party ends it earlier under this clause, will end when the Project is completed and the Collaborator has paid the Total Fees.

Either party may end this Agreement immediately by giving notice to the other party if that other party:

breaches any provision of this Agreement and fails to remedy the breach within 30 days after receiving notice requiring it to do so; or

breaches a material provision of this Agreement where that breach is not capable of remedy.

The Collaborator must notify CSIRO immediately if:

there is any change in the direct or indirect beneficial ownership or control of Collaborator which would affect its ability to comply with its obligations under this Agreement;

it disposes of the whole or any part of its assets, operations or business other than in the ordinary course of business;

it ceases to carry on business;

it ceases to be able to pay its debts as they become due;

any step is taken by a mortgagee to take possession or dispose of the whole or any part of its assets, operations or business;

any step is taken to enter into any arrangement between Collaborator and its creditors;

any step is taken to appoint a receiver, a receiver and manager, a trustee in bankruptcy, a liquidator, a provisional liquidator, an administrator or other like person of the whole or any part of its assets or business; or

where Collaborator is a partnership, any step is taken to dissolve, or which has the effect of dissolving, that partnership.

*CSIRO may terminate this Agreement with immediate effect by giving notice to the Collaborator if any event referred to in **clause 12.3** happens to Collaborator.*

AFTER END OF AGREEMENT

After the end of the Agreement:

unless the Collaborator has ended the Agreement as a result of breach by CSIRO, the Collaborator must pay CSIRO for work done and expenses incurred up to the end of the Agreement;

if CSIRO ends it for breach by the Collaborator, CSIRO retains ownership of all Project Results and is not bound to grant the licence referred to in clause 7 (Project Results);

each party's (the 'first party') right to use Confidential Information of the other party ceases and the first party must immediately at the other party's request and option:

return to the other party;
destroy and certify in writing to the other party the destruction of; or
destroy and permit the other party to witness the destruction of
all of the other party's Confidential Information in the first party's possession or control;

clauses 7 **(Project Results)**, 8 **(Confidentialty)** 10 **(Warranties and Exclusions),** 11 **(Liability),** 11 **(Dispute Resolution) and** 15 **(Use of Names and Publication) continue; and**

accrued rights or remedies of either party are not affected.

UNEXPECTED EVENTS

*'**Unexpected Event**' affecting a party means anything outside that party's reasonable control, including but not limited to, acts or omissions of the other party, fire, storm, flood, earthquake, war, transportation embargo or failure or delay in transportation, act or omission (including laws, regulations, disapprovals or failures to approve) of any third person (including but not limited to, subcontractors, customers, governments or government agencies).*

*If an Unexpected Event affecting a party precludes that party ('**precluded party**') partially or wholly from complying with its obligations under this Agreement then:*

as soon as reasonably practicable after that Event arises, the precluded party must notify the other party of the Unexpected Event; and

to the extent and for the period that the precluded party is precluded by the Unexpected Event from complying with its obligations under this Agreement, those obligations will be suspended.

This clause does not apply to any obligation to pay money.

USE OF NAMES AND PUBLICATION

The Collaborator must obtain written consent from CSIRO before it:

uses the names Commonwealth Scientific and Industrial Research Organisation or CSIRO or any trademark or logo of CSIRO; or

uses the Report as a means of product endorsement by CSIRO.

NOTICES

A party notifying or giving notice under this Agreement must give notice in writing, addressed to the other party's contact specified in the Details.

GENERAL

Divisional Limitation: *The Client acknowledges that CSIRO's obligation to carry out the Project is limited to an obligation to do so using the resources of the Division.*

Relationships:

This Agreement does not create a relationship of employment, agency or partnership between the parties; and

The rights and obligations of each party under this Agreement are several, not joint or joint and several.

Further Action: *Each party must do or cause to be done all things necessary or desirable to give effect to, and refrain from doing things that would hinder performance of, this Agreement.*

Assignment: *A party must not assign or attempt to assign or otherwise transfer any right arising out of this Agreement without the written consent of the other party.*

Waiver: *The failure of a party at any time to insist on performance by the other party of any obligation under this Agreement is not a waiver of its right:*

to insist on providing of, or to claim damages for breach of, that obligation unless that party acknowledges in writing that the failure is a waiver; and

at any other time to insist on performance of that or any other obligation of the other party under this Agreement.

Severability: *If part or all of any clause of this Agreement is illegal or unenforceable it will be severed from this Agreement and will not affect the continued operation of the remaining provisions.*

Costs: *The Licensee must pay all stamp duty and its own legal costs associated with preparing and finalising this Agreement.*

Entire Agreement*: This Agreement:*

is made up of the Details, the Project Plan and these terms;

records the entire Agreement between the parties and supersedes all earlier Agreements and representations by the parties about its subject matter;

may only be altered in writing signed by both parties.

Inconsistency: *To avoid inconsistent provisions applying:*

no confirmation, shipment or delivery docket, invoice, terms and conditions of supply or other document issued by or on behalf of the Collaborator about the Project will vary this Agreement; and

if there is any inconsistency between the Project Plan and the Details or the Terms, the Project Plan will prevail to the extent of the inconsistency.

Governing Law: *This Agreement is governed by the Governing Law.*

CSIRO

Terms for Collaborative Research Agreement

Appendix 3: Minutes of first Project Consultative Group meeting held 7th February 2000.

SRDC Project CTA045 – Consultative Group meeting

"Improving CCS in the wet tropics via block-specific monitoring of N in cane delivered to the mill."

Date: Monday, 7 February 2000

Place: Sugar North Ltd, Cairns

Meeting opened at 12.05 pm.

Attendance: Russell Muchow (chair), Robert Rossi, Basil Micale, Scott Grimley, Robert Sutherland, Les Robertson. *Ex officio* Brian Keating, Tony Webster, Steve Staunton.

Apologies: Alan Cole, Ray McDowall, John Reghenzani.

Introduction: The chairman, Russell Muchow welcomed members to this first meeting, and introduced the project.

Background: Brian Keating presented the background to the project, which arose from previous studies at Bundaberg and elsewhere.

(Presentation attached)

Role of Consultative Group: The role of the Consultative Group was discussed and the members **endorsed** the tabled Role Statement with the following amendments:

1. Point 3 replace *adaptability* with *useability*; and
2. Addition of Point 5, *'To advise project team on industry issues concerned with this project CTA045.'*

(Revised Role Statement attached)

Work Plan: The work plan of the project team was discussed, and **endorsed** by the committee, with the proviso that the last paragraph of document 'Grower Working Groups' be amended to include collaboration with BSES extension service as well as with Productivity Officers within group processes.

(Revised document 'Grower Working Groups' attached)

General Operational issues:

Data access – General Managers of Sugar North mills have agreed with the principle of providing data to this project. The Consultative Group **endorsed** the text of the draft letter to go to each mill (requesting data), with the addition of the statement about;

1. the endorsement of this Consultative Group and its role in information management.

(Draft letter to Mill Managers attached)

Confidentiality – The letters from Scott Grimley (Sugar North) to SRDC, and the reply from SRDC were **noted.** The Consultative Group **endorsed** the proposal that the Group could recommend whether any reports emanating from this project should be restricted (eg in Annual Reports, etc) because of sensitivities on economic or environmental grounds.

(Letters (1) from Scott Grimley to SRDC, and (2) reply from SRDC attached)

Project Communication Strategies -

ACTION: Tony Webster to discuss with Bob Rossi the possible role and contribution of FarmBi$ in this project CTA045, and to jointly develop a proposal for consideration at the next meeting (May).

ACTION: Tony Webster to talk with BSES about displays on this project at BSES Field Days, and to consider displays at the Australian Sugar Convention and ASSCT Conference.

ACTION: Communication to appear as an agenda item for each subsequent meeting of the Consultative Group.

Managing demand for service from growers – The Consultative Group **endorsed** this project as a research activity rather than as a project to service requests from individual industry representatives. The connection with Productivity Officers and BSES extension should serve as the conduit for information flow and extension to Industry in general. The need for extension of information on NIR in general (as well as its use in amino-N monitoring) was recognised by the members.

ACTION: The Project Team to prepare a summary for the next meeting on the important factors likely to impact on amino-N in cane (eg mill mud, fertiliser N, trash retention, season, variety etc), and the importance of interpretation of predicted values. This would be used in early communication initiatives.

ACTION: Scott Grimley to review available information from other Sugar North mills with NIR.

Other Business:

Steve Staunton gave a brief update on development of predictions based on correlation with measured amino-N (currently with r^2 of 0.89). The Consultative Group **endorsed** the use of data from Tablelands cane as well as Mossman coastal cane in development of amino-N predictions in this project.

ACTION: Steve Staunton to give an update of development of NIR predictions of amino-N at next meeting (May).

The Consultative Group **endorsed** Russell Muchow as continuing chairman of this group.

Next Meeting:

Date – Thursday 11 May 2000, at 12.00 noon

Place – Sugar North Ltd, McLeod Street Cairns.

Meeting closed at 2.15 pm.

ROLE STATEMENT

PROJECT CONSULTATIVE GROUP –

Delivering block-specific N management via Mill N monitoring

(Short title: Mill N Monitoring Project)

1. To deliver and implement information management protocols to ensure effective delivery of project research results.

2. To facilitate constructive consideration and discussion to enhance the understanding and application of research information within the industry to optimise the potential of industry resources.

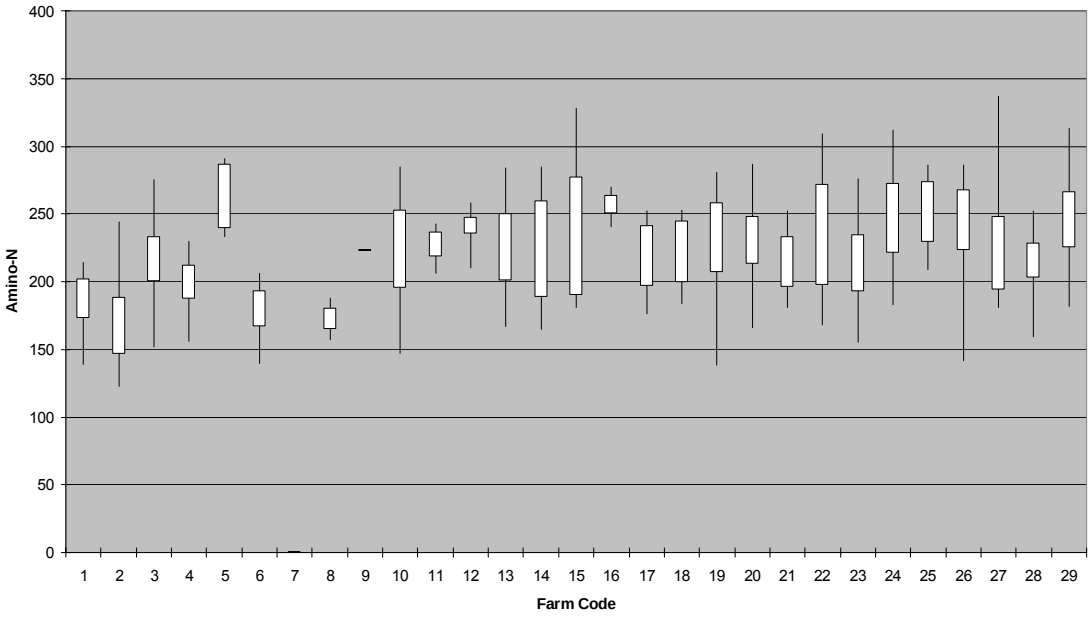
3. To provide feedback on focus, methodology and interpretation of research to support its usability to industry needs.

4. To advise Project Team on Industry communication issues.

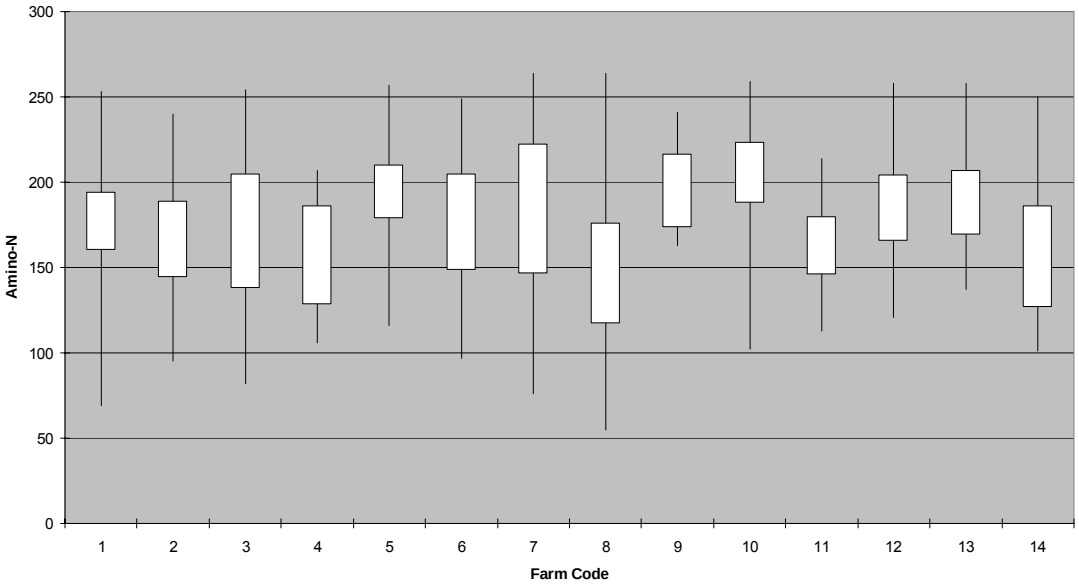
5. To advise Project Team on Industry issues concerned with this project CTA045.

Appendix 4: Baseline Amino-Nitrogen data for farms involved in project Grower Working Groups.

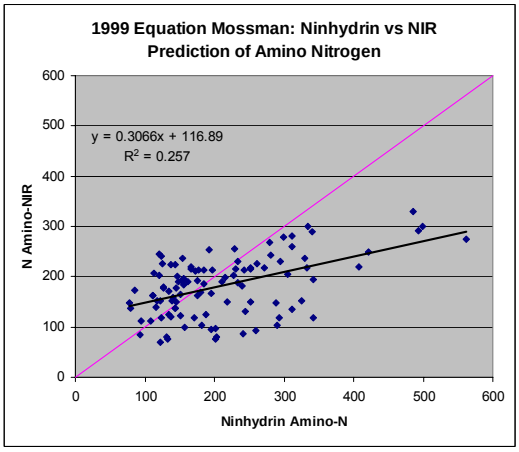
1999 Mulgrave Grower Working Group Farm Box (25 to 75 percentile) and Whisker (Min, Max)

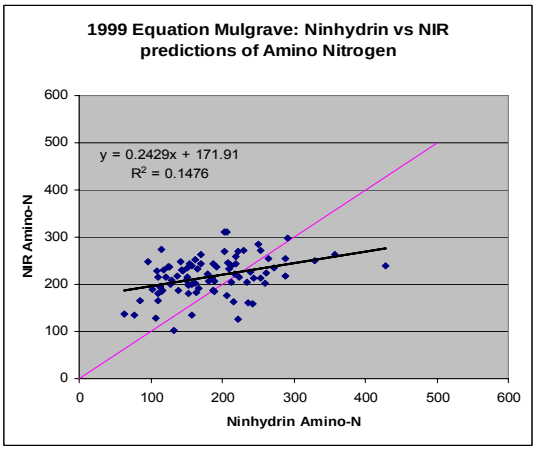


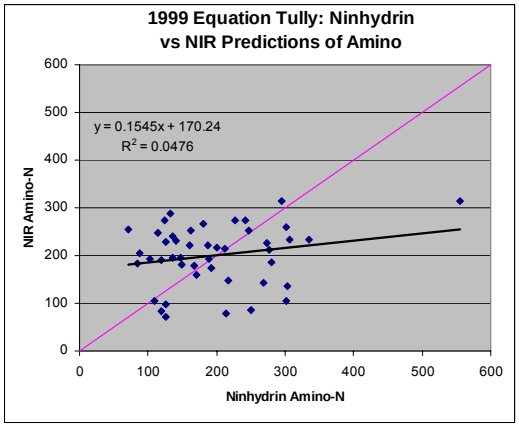
Mossman 1999 Grower Working Group Farm Amino-N Box (25 to 75 Percentile) and Whisker (Min, Max)



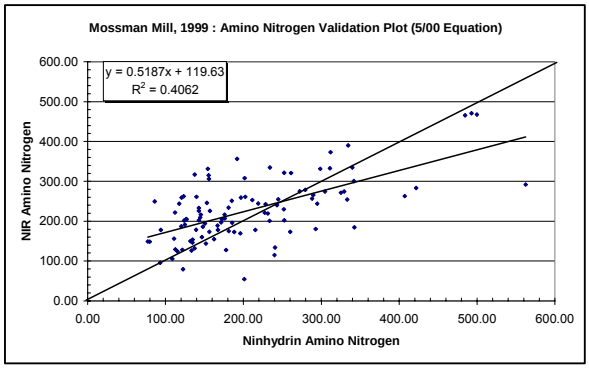
Appendix 5: Validation plots of 1999 equation tested with samples taken during 1999 from Sugar North mills (no data for South Johnstone).

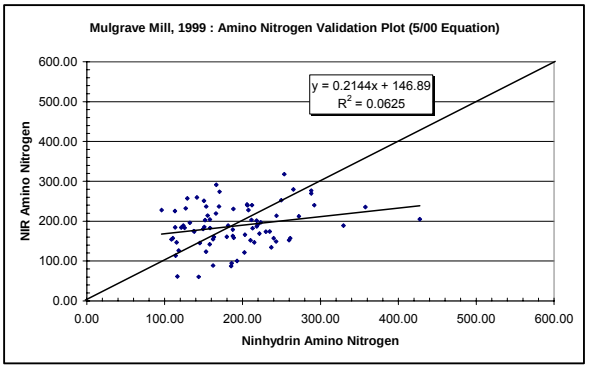


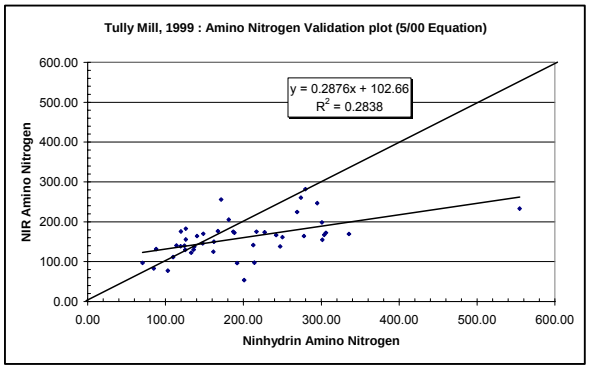


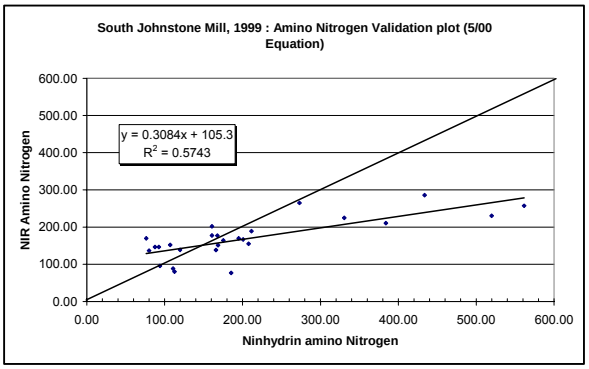


Appendix 6: Validation plots of 5/00 Equation for four Sugar North Mills.

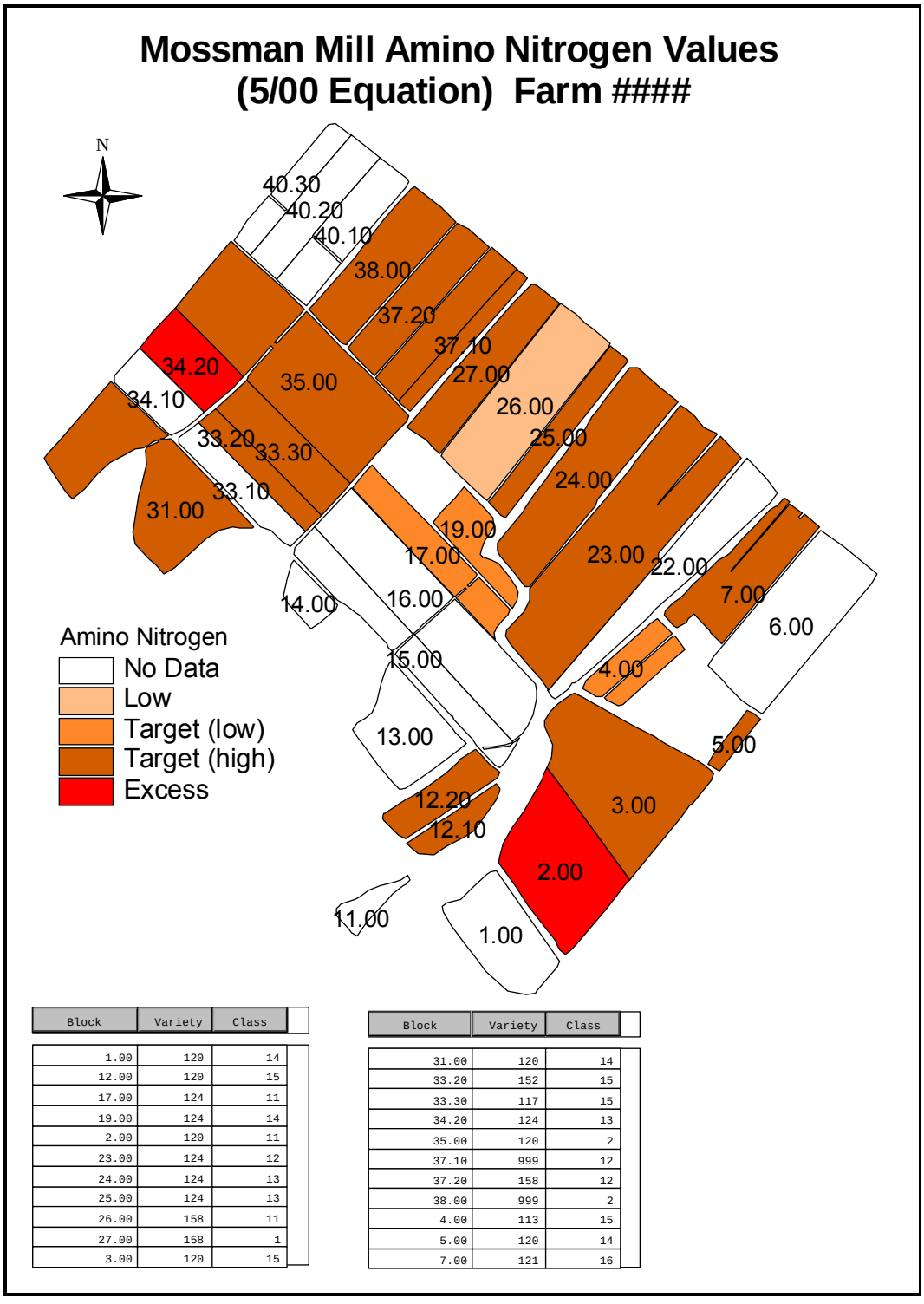






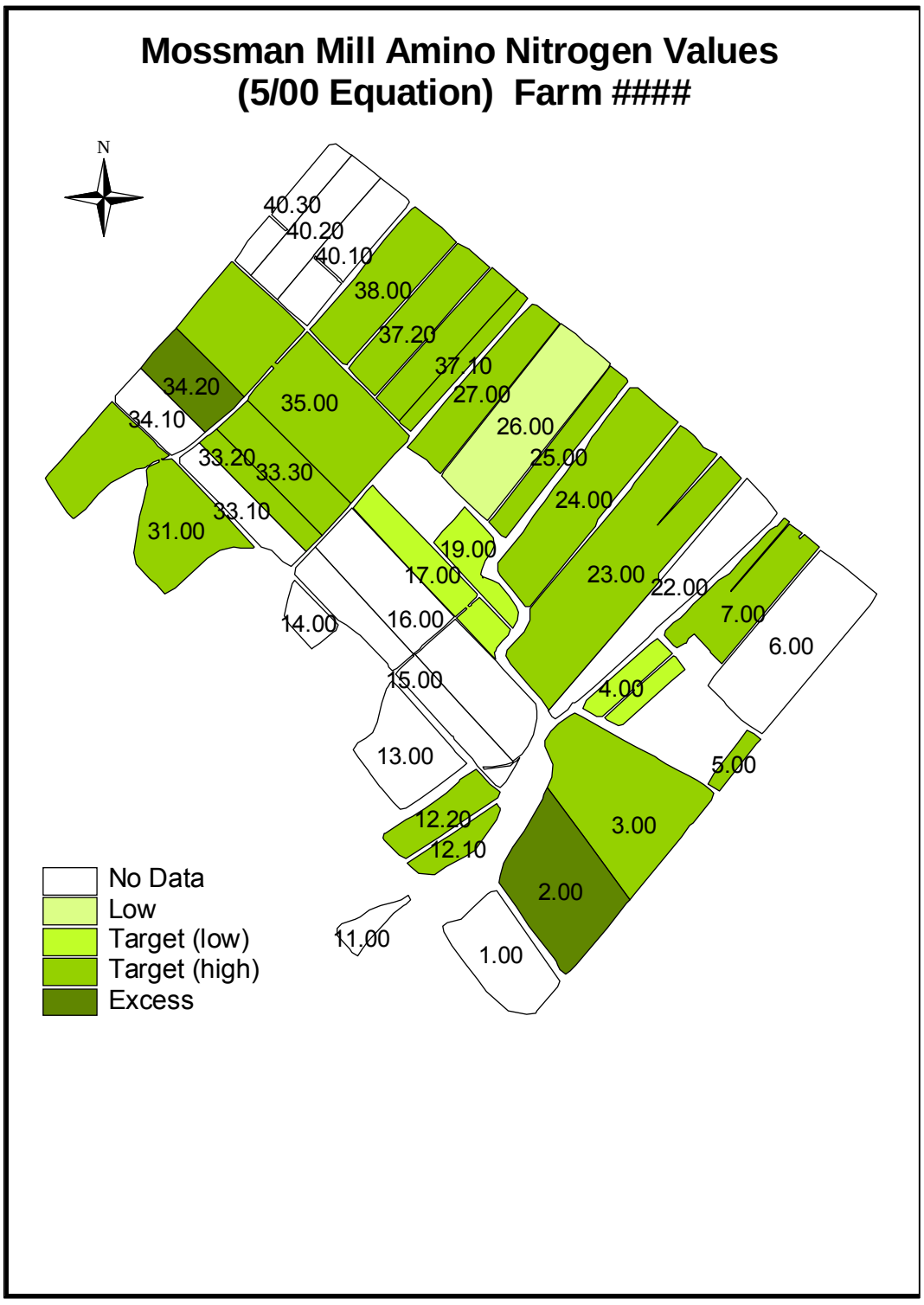


Appendix 7: Examples of data presentation formats for end-of-season reporting of amino-N.

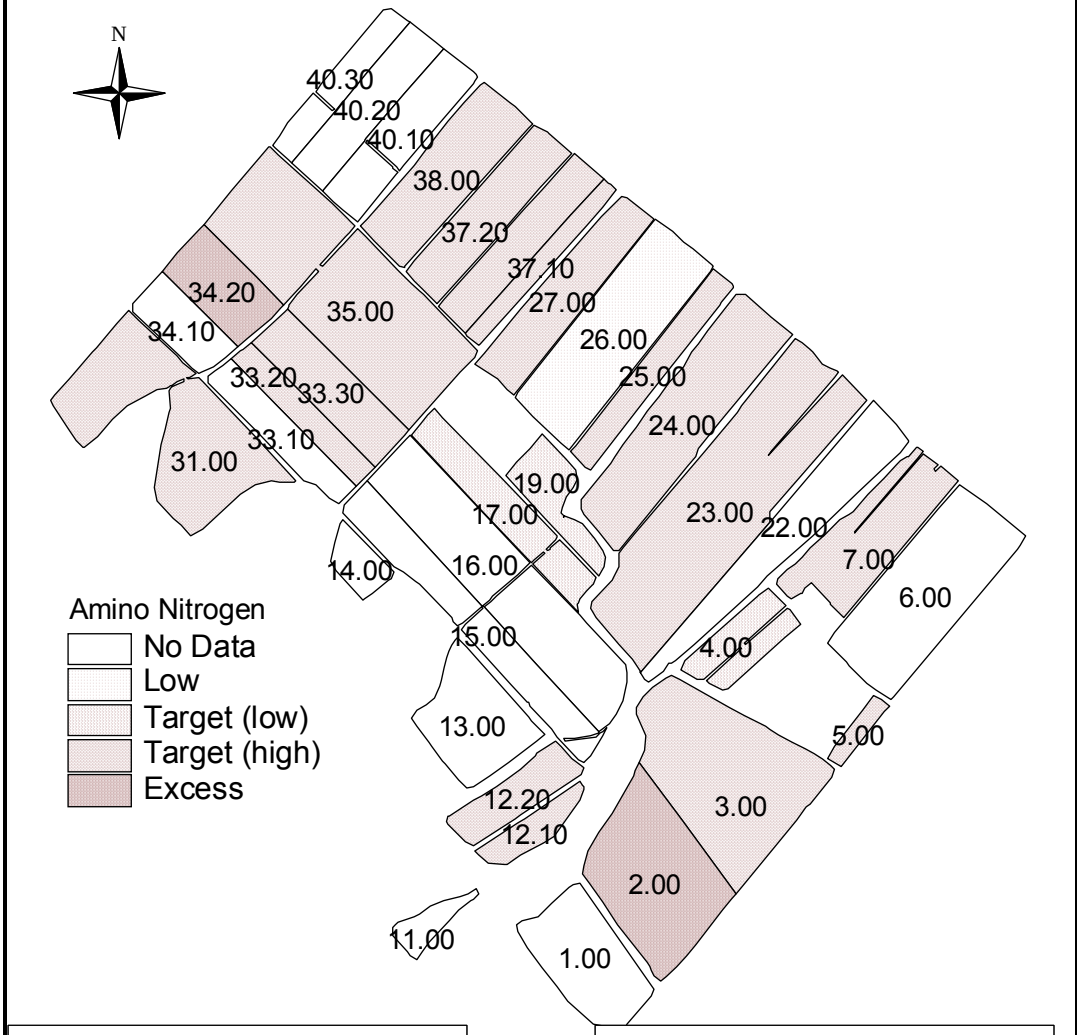


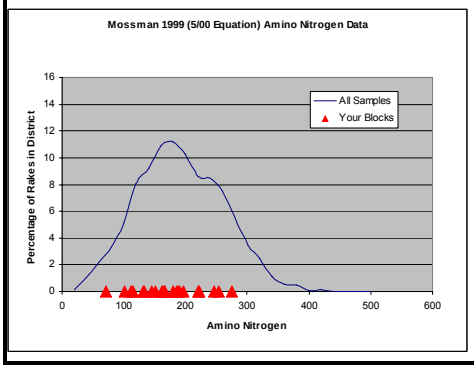
Block	Variety	Class
1.00	120	14
12.00	120	15
17.00	124	11
19.00	124	14
2.00	120	11
23.00	124	12
24.00	124	13
25.00	124	13
26.00	158	11
27.00	158	1
3.00	120	15

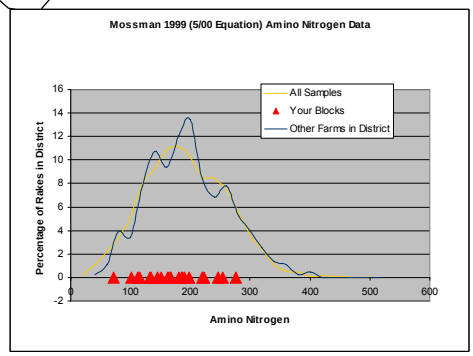
Block	Variety	Class
31.00	120	14
33.20	152	15
33.30	117	15
34.20	124	13
35.00	120	2
37.10	999	12
37.20	158	12
38.00	999	2
4.00	113	15
5.00	120	14
7.00	121	16


Mossman Mill Amino Nitrogen Values
(5/00 Equation) Farm ####
N
40.30
40.20
40.10
38.00
37.20
37.10
27.00
26.00
25.00
24.00
23.00
22.00
7.00
6.00
4.00
5.00
3.00
2.00
1.00
11.00
12.10
12.20
13.00
15.00
16.00
14.00
17.00
19.00
31.00
33.10
33.30
33.20
35.00
34.10
34.20
No Data
Low
Target (low)
Target (high)
Excess

Mossman Mill Amino Nitrogen Values (5/00 Equation) Farm



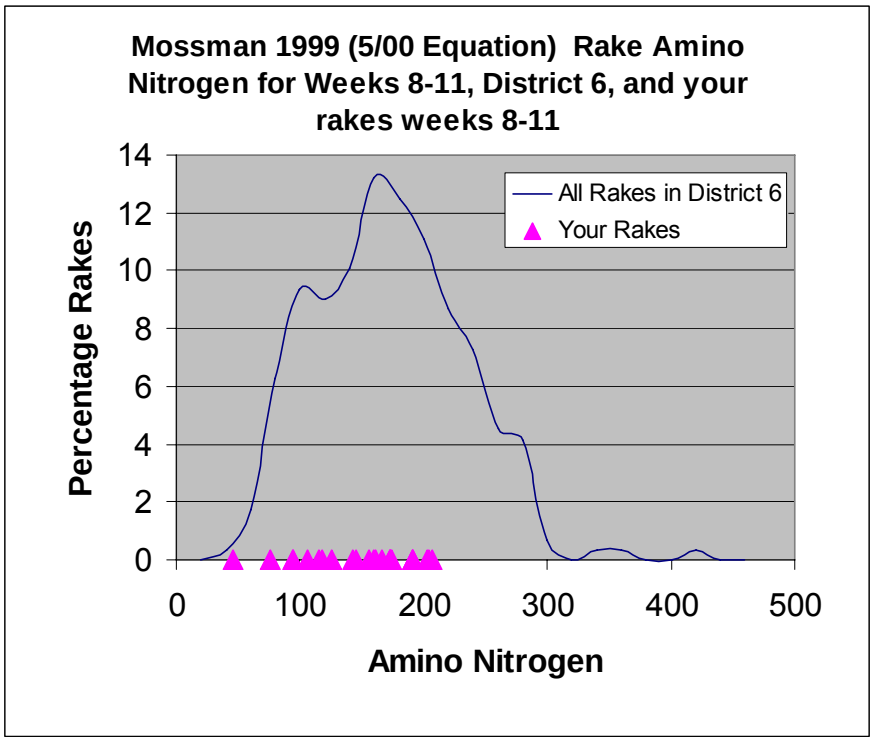


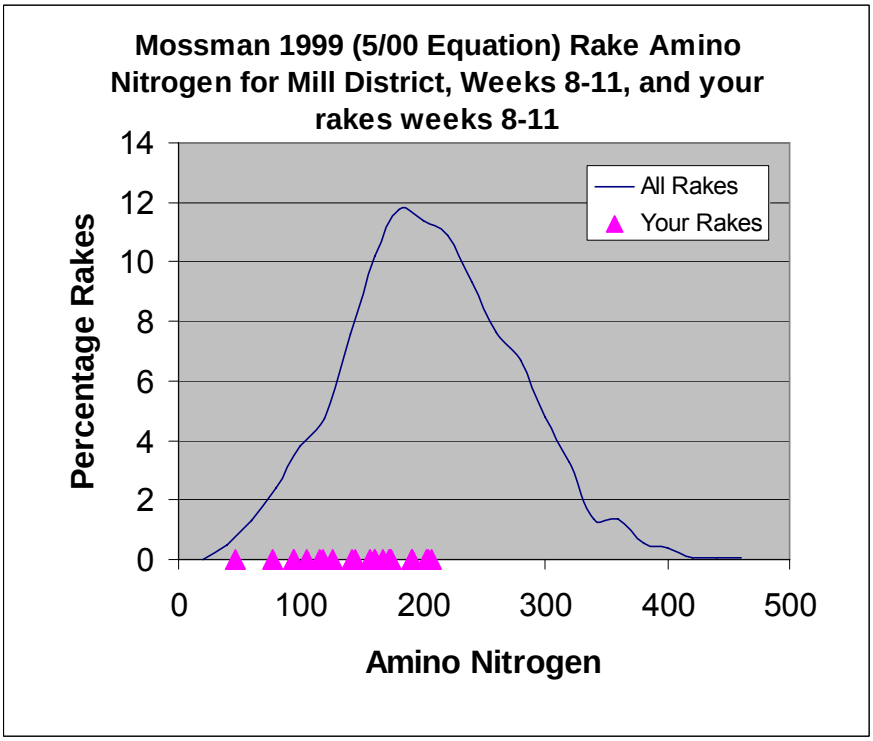


Appendix 8: Examples of data presentation formats for in-season reporting of amino-N.

Rake	Farm	Block	Week	Amino_N	Variety	Class	CCS	Tonnes
6960	####	23.00	8	172.69	124	12	9.6	85.39
6983	####	23.00	8	159.88	124	12	10.1	66.59
7040	####	23.00	8	202.83	124	12	10.1	59.4
7041	####	23.00	8	204.02	124	12	10.3	59.51
7078	####	23.00	8	173.14	124	12	9.9	74.91
7091	####	23.00	8	190.65	124	12	10.1	94.99
7144	####	7.00	8	166.65	121	16	13.5	39.08
7150	####	7.00	8	191.35	121	16	14	49.57
7151	####	5.00	8	144.83	120	14	12.6	17.97
7152	####	17.00	8	114.97	124	11	10.5	67.93
7198	####	23.00	9	207.01	124	12	10.1	57.68
7199	####	23.00	9	191.23	124	12	10.3	56.97
7246	####	23.00	9	172.44	124	12	11.1	18.37
7247	####	24.00	9	126.3	124	13	10.7	56.31
7248	####	24.00	9	173.51	124	13	10.9	57.25
7292	####	24.00	9	160.51	124	13	10.6	88.77
7293	####	24.00	9	76.39	124	13	9.5	75.51
7294	####	25.00	9	142.46	124	13	9.8	47.54
7352	####	25.00	9	156.2	124	13	9.7	67.34
7353	####	26.00	9	45.77	158	11	10.6	55.14
7354	####	26.00	9	93.59	158	11	10.7	43.74
7617	####	19.00	9	118.19	124	14	12.4	18.32
7618	####	19.00	9	105.37	124	14	11.3	18.08
7620	####	26.00	9	93.8	158	11	10.8	17.44

Week	Farm	Block	Rake	Variety	Class	CCS	Tonnes	Amino_N	Weighted Block Ave Amino-N
8	####	5.00	7151	120	14	12.6	17.97	144.83	
									Target (low) 145
8	####	7.00	7144	121	16	13.5	39.08	166.65	
8	####	7.00	7150	121	16	14	49.57	191.35	
									Target (high) 180
8	####	17.00	7152	124	11	10.5	67.93	114.97	
									Target (low) 115
9	####	19.00	7617	124	14	12.4	18.32	118.19	
9	####	19.00	7618	124	14	11.3	18.08	105.37	
									Target (low) 112
8	####	23.00	6960	124	12	9.6	85.39	172.69	
8	####	23.00	6983	124	12	10.1	66.59	159.88	
8	####	23.00	7040	124	12	10.1	59.4	202.83	
8	####	23.00	7041	124	12	10.3	59.51	204.02	
8	####	23.00	7078	124	12	9.9	74.91	173.14	
8	####	23.00	7091	124	12	10.1	94.99	190.65	
9	####	23.00	7198	124	12	10.1	57.68	207.01	
9	####	23.00	7199	124	12	10.3	56.97	191.23	
9	####	23.00	7246	124	12	11.1	18.37	172.44	
									Target (high) 186
9	####	24.00	7247	124	13	10.7	56.31	126.3	
9	####	24.00	7248	124	13	10.9	57.25	173.51	
9	####	24.00	7292	124	13	10.6	88.77	160.51	
9	####	24.00	7293	124	13	9.5	75.51	76.39	
									Target (low) 133
9	####	25.00	7294	124	13	9.8	47.54	142.46	
9	####	25.00	7352	124	13	9.7	67.34	156.2	
									Target (low) 151
9	####	26.00	7353	158	11	10.6	55.14	45.77	
9	####	26.00	7354	158	11	10.7	43.74	93.59	
9	####	26.00	7620	158	11	10.8	17.44	93.8	Low 71


Mossman 1999 (5/00 Equation) Rake Amino Nitrogen for Weeks 8-11, District 6, and your rakes weeks 8-11
Percentage Rakes
14
12
10
8
6
4
2
0
0
100
200
300
400
500
Amino Nitrogen
All Rakes in District 6
Your Rakes


Mossman 1999 (5/00 Equation) Rake Amino Nitrogen for Mill District, Weeks 8-11, and your rakes weeks 8-11
Percentage Rakes
14
12
10
8
6
4
2
0
0
100
200
300
400
500
Amino Nitrogen
All Rakes
Your Rakes

Appendix 9: Guidelines for amino-N data.

CSIRO researchers have been working on ways in which sugarcane growers can improve the precision of their nitrogen management. This work is aimed at reducing fertiliser costs and the risk of over fertilisation lowering CCS or contaminating ground or surface waters. The work is supported by the Sugar Research and Development Corporation (SRDC).

What is Amino Nitrogen?

Amino nitrogen is a measure of the nitrogen associated with amino acids in cane stems. Nitrogen is taken up and stored by sugarcane in the cane stem as amino nitrogen. When the growing plant requires stored nitrogen, it is transported from these amino acids to where the nitrogen is required.

Why measure Amino-N?

Amino nitrogen is measured in cane stems as supplied to the mill to provide a guide to the nitrogen status of the harvested crop. Measuring the level of nitrogen in amino acids in the cane juice (referred to as amino-N) provides this information.

Measuring amino-N is a very good method for picking up situations where nitrogen is in excess because sugarcane stores its excess nitrogen as amino-N.

How is Amino-N measured?

Amino-N is usually determined through a laboratory analysis of the cane juice called the *ninhydrin reactive test*. In a sugar mill, NIR is able to measure amino-N of the cane juice instead. Using NIR allows all rakes of cane delivered to the mill to be measured for amino-N.

What does Amino-N tell me?

Amino-N provides a guide as to the nitrogen status of the harvested crop. The Amino-N measurement is a reflection of the balance between nitrogen supplied to the crop, and that crops demand for nitrogen.

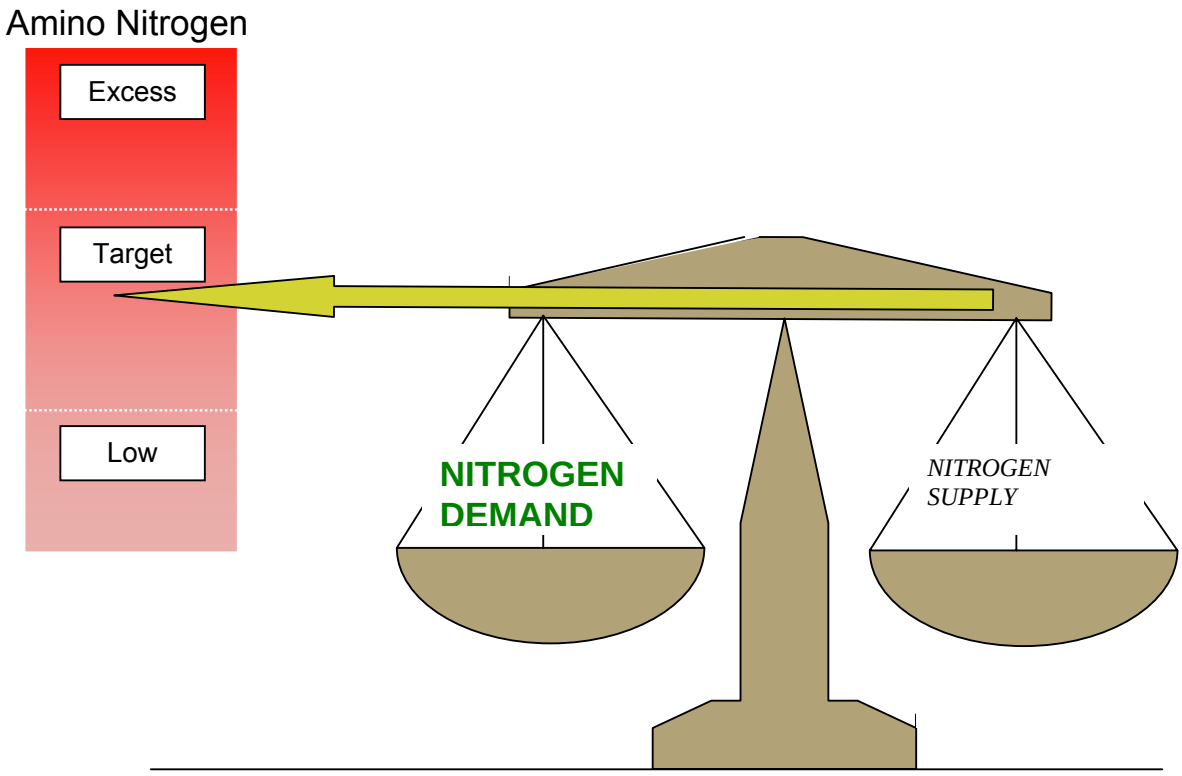
Amino nitrogen measures the balance between crop demand for nitrogen and nitrogen supply. When amino-N is high, it is an indicator the nitrogen supplied to the crop was in excess of crop demand. When amino-N is low, it is an indicator the crops demand for nitrogen was not met sufficiently by the nitrogen supply.

The factors that influence crop demand for nitrogen and supply of nitrogen to a crop are the same factors that influence amino-N.

The Amino-N ‘balance’

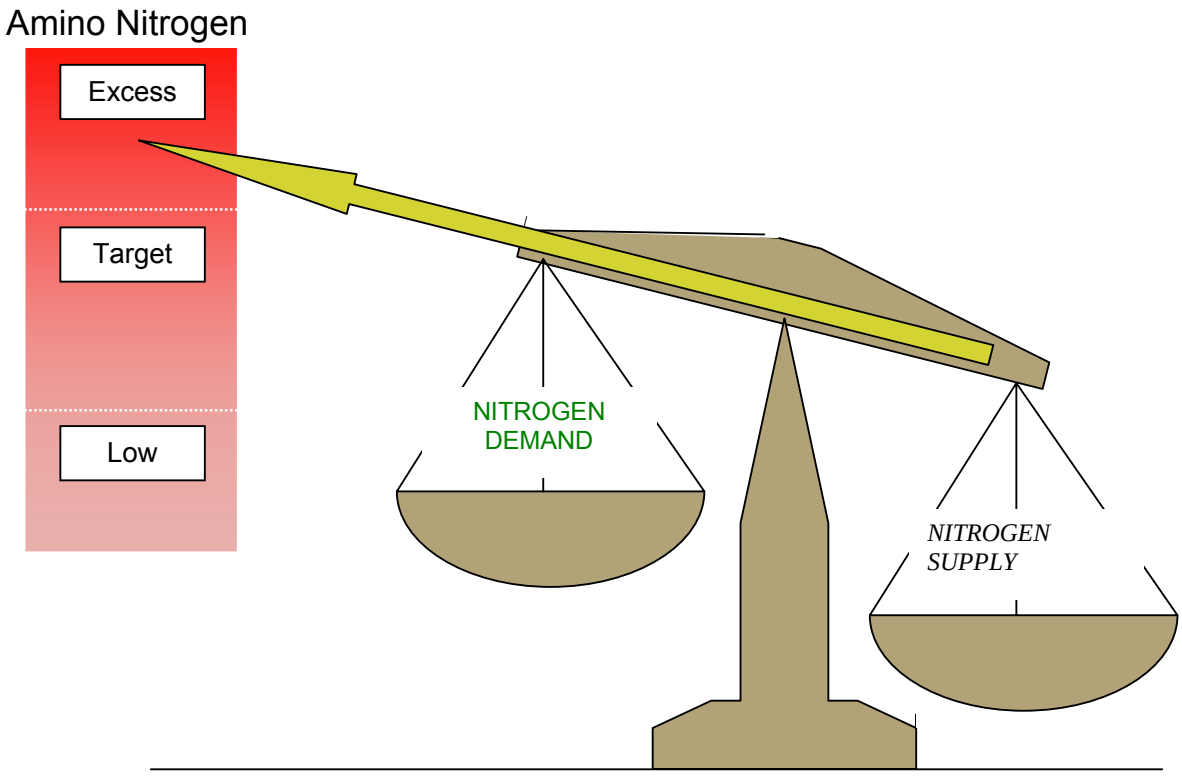
A balance can be used represent the amino-N measurement. On one side of the balance is nitrogen supply to the crop, the greater the nitrogen supplied, the further down the balance moves on this side. Nitrogen supply is balanced against crop demand for nitrogen. Larger crops with a greater crop demand cause the balance to move down further on this side of the balance. When crop demand and nitrogen supplied are in balance, amino-N is in the target range.

Target Range Amino Nitrogen



actors that influence **NITROGEN DEMAND**	Factors that influence **NITROGEN SUPPLY**
• Crop Size – weather – water logging – crop lodging – location – ratoon age – pest damage – disease incidence – other nutritional constraints • Possibly variety	• N fertiliser inputs (rates, forms, timing and methods) • Soil N sources (soil type and history) – mineral N residues – mineralisation • Mill wastes • Crop residues • Other minor N sources (rainfall, fixation) • N loss processes – leaching, denitrification, run off, volatilization

Excess Amino Nitrogen

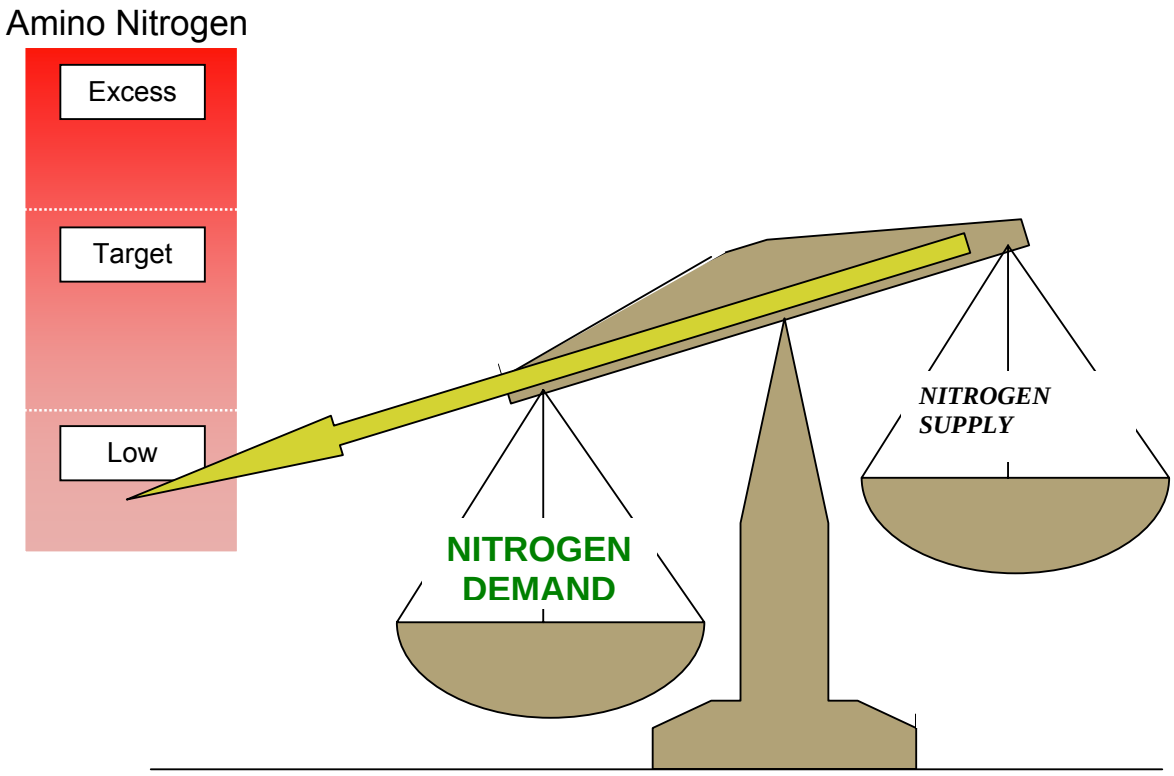


When amino-N is in the excess range it indicates nitrogen supply out-weighed nitrogen demand by the crop. This situation means there may have been nitrogen fertiliser wasted by putting on more than crop demand. Excess amino-N also suggests there may be a risk of nitrogen leaving the farming system, possibly contaminating surface or ground waters. Excess amino-N can also result when crop demand for nitrogen is very low, as occurs when a very small crop is grown. Nitrogen inputs for two crops may be similar, but a small crop will return a higher amino-N value because of a lower nitrogen demand.

Those factors that increase nitrogen supply are usually responsible for increasing amino-N. Nitrogen fertiliser inputs, both for the current crop and previous crops, have a large impact on nitrogen supply. The rate, form, timing and application methods of nitrogen fertilisers can each influence supply. The more available nitrogen fertiliser is to sugarcane, the more likely it will influence the nitrogen supply side of the balance.

The soil also has a major impact on nitrogen supply, and consequently amino-N. Over time microorganisms in the soil cause organic nitrogen (unavailable to plants) to be mineralised into forms sugarcane can take up. When soil is left fallow for a period of time before planting more mineralisation occurs, increasing the amount of nitrogen supplied to the crop. Legume crops (beans, lab lab) are able to convert nitrogen in the atmosphere into forms that plants can use. When a legume crop is grown in the fallow extra nitrogen is added to the soil. Plant crops following legumes will have an increased nitrogen supply, which could raise amino-N values.

Low Amino Nitrogen



Low amino-N indicates crop demand for nitrogen outweighed nitrogen supply. Larger crops have a higher demand for nitrogen than smaller crops. Low

A range of things can be responsible for nitrogen supply to a crop being low. Simply, not enough nitrogen may have been applied, causing low amino-N values. Alternatively sufficient nitrogen may have been applied, but the crop was unable to access it for some reason. These reasons can include nitrogen being lost to the atmosphere, ground or surface water before the crop had an opportunity to take it up, nitrogen being applied in an unusable form, time or place, or soil microorganisms consuming nitrogen before the crop had a chance to take it up.

Appendix 10: Report on the impact of composition of cane supply on the amino-N monitoring technique.

Impact of extraneous matter constituents on amino nitrogen

Experimentation

Experimentation has been conducted to examine the effect of the individual extraneous matter components soil, cabbage (tops), trash (dead leaf) and leaf (green leaf) on amino nitrogen. The amino-N of clean cane and clean cane +2% or +5% of each extraneous matter component was determined from experimental plots with two nitrogen application rates, 0 and 180 kg N/ha. All treatments are from an experiment with three reps of Q124 plant cane harvested at thirteen months old on 1st September 1997.

0 kg N/ha	180 kg N/ha
Clean cane	Clean cane
Clean cane + 2% soil	Clean cane + 2% soil
Clean cane + 5% soil	Clean cane + 5% soil
Clean cane + 2% trash	Clean cane + 2% trash
Clean cane + 5% trash	Clean cane + 5% trash
Clean cane +2% leaf	Clean cane +2% leaf
Clean cane +5% leaf	Clean cane +5% leaf
Clean cane + 2% cabbage	Clean cane + 2% cabbage
Clean cane + 5% cabbage	Clean cane + 5% cabbage

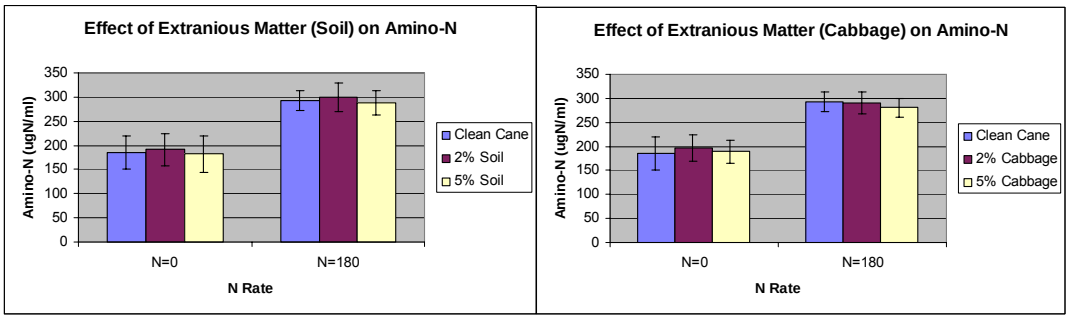
Table 1: Treatments of extraneous matter experiment.

Clean cane, cabbage, leaf and trash were shredded separately using a Jeffco cutter grinder. A clean cane sample was pressed with a carver press and the juice collected for amino-N analysis. Each extraneous matter component was then added at 2% and 5% levels by weight to clean cane and pressed with juice collected for analysis.

Results

Amino-N analyses of each treatment suggest soil and cabbage do not influence amino-N at the 2% and 5% levels. Both trash (dead leaf) and leaf (green leaf) slightly increased amino-N at both levels of extraneous matter, with 5% of each component increasing amino-N more than at the 2% level, as shown in figure 1.

(a) (b)



(c) (d)

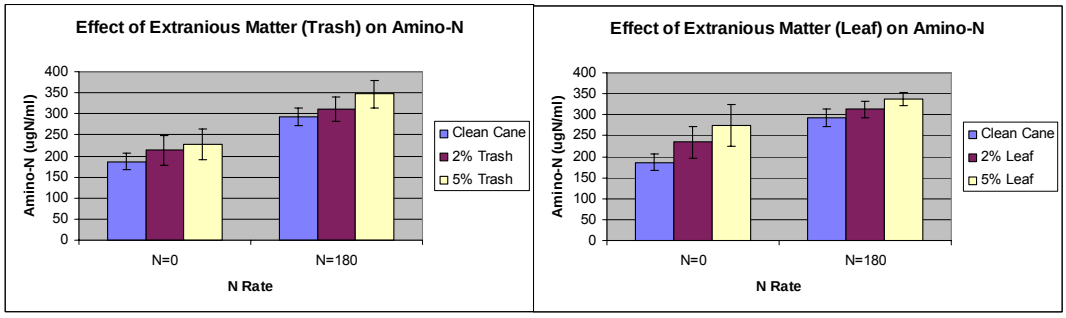


Figure 1: Influence of extraneous matter components on amino-N. (a) Soil, (b) Cabbage, (c) Trash, (d) Leaf.

All four treatments showed an increase in amino-N levels at the higher nitrogen application rate (180 kg N/ha compared with 0 kg N/ha). The slight decrease in amino-N levels in the 5% soil (a) and 5% cabbage (b) treatments at the high N application rate are insignificant, attributable to acceptable lab error (293 – 280 ug/ml and 293 – 288 ug/ml respectively).

There is a suggestion trash (dead leaves) increase amino-N levels when mixed with clean cane, particularly when 5% trash is added at the higher N application rate (c). When leaf (green leaves) are mixed with clean cane at the 5% level amino-N is increased significantly at both 0 and 180 kg N/ha application rates (d).

There is a suggestion that both trash and leaf increase amino-N when mixed with clean cane. Both graphs show a stepwise increase from clean cane to 2% mixture to 5% mixture at both N application rates. This increase in amino-N does not appear to occur when either soil or cabbage are mixed with clean cane.

Interpretation

There are two immediate possibilities as to why trash and leaf may increase amino-N levels as they are added to clean cane. Firstly, amino-N could be in high levels in the leaf, and being squeezed out with the carver pressing operation. Alternatively, trash and leaf mixed with clean cane may inhibit the ability of the carver press to extract all the cane juice. If all the amino-N can be squeezed out, yet less of some other juice constituents (particularly water), this would have the effect of increasing the concentration of amino-N in the juice. Possibly both these actions are occurring.

This experiment added shredded extraneous matter components to already shredded cane; therefore the effect of extraneous matter on the operation of the shredder in the factory, and

its impact on amino-N cannot be quantified. It is understood high levels of extraneous matter may affect the ability of the shredder to effectively pulverize cane and therefore extract juice.

'Typical' Extraneous Matter Levels

Mulgrave mill sampled rakes for extraneous matter through the season via 'grabbing' a sample from random rakes and separating the sample into tops (cabbage), trash (leaf and trash), suckers and cane. Cane is then sub-divided into borer damaged cane, other damaged cane and sound cane. Levels of extraneous matter were then averaged from 43 rakes sampled during the 2000 season and are presented here. Box 'n whisker presentation shows the middle 50% (25 to 75 percentile) of samples (box) and upper and lower levels (end of each whisker).

Figure 2: Levels of extraneous matter components averaged from 43 rakes at Mulgrave Mill during 2000 season.

Significantly tops and trash levels monitored from Mulgrave mill can be much greater than the 2% and 5% levels tested in the previously reported extraneous matter experiment. There may also be a significant amount of suckers and damaged cane in rakes delivered to Mulgrave Mill, their effect on amino-N levels is not known.

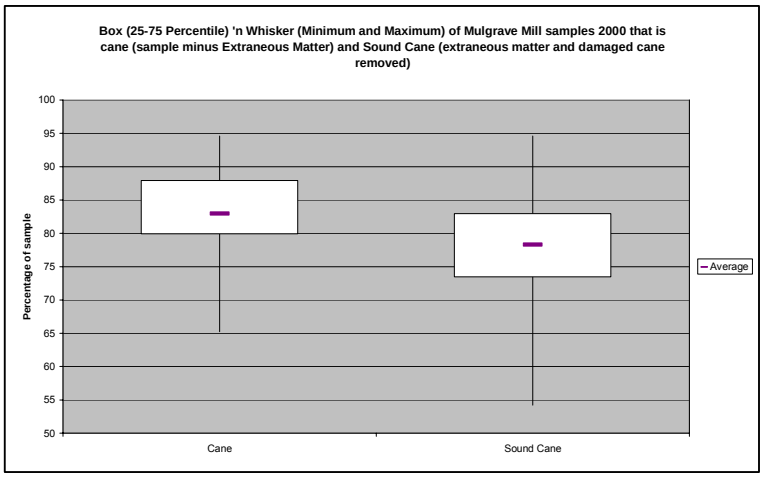


Figure 3: Levels of cane (total minus tops, trash and suckers) and sound cane (cane minus damaged cane) averaged from 43 rakes at Mulgrave Mill during 2000.

Figure 3 shows levels of sound cane delivered to the mill averaged 78% sampled from 43 rakes during 2000, the remainder is various extraneous matter components. The experimentation above added only 2% or 5% extraneous matter levels. The experimentation also added each extraneous matter component individually, the effect of combining extraneous matter components together with clean cane is not known.

As the NIR system used to monitor amino-N universally from each rake at the mill is currently unable to provide a satisfactory measurement, the effect of components of cane on the NIR system of monitoring amino-N has not been determined. It is expected extraneous matter components would have two effects on the ability of NIR to measure amino-N. Firstly, each extraneous matter component would potentially have a direct effect on amino-N as outlined in the experimentation. Additionally, extraneous matter tends to influence the ability of the shredder in the mill to work efficiently. Also, high levels of extraneous matter can influence the presentation of shredded cane to the NIR window in the number one mill feed chute.

It is anticipated further experimentation will be conducted during the 2001 crushing season to determine what effect higher levels of extraneous matter has on amino-N, particularly trash and green leaf. Experimentation is also planned on how the presentation of cane to the NIR window may influence the ability of NIR to measure amino-N.

Appendix 11: Report on an assessment of the interpretation of relationships between CCS and amino-N levels in cane supply.

Data analysis – Amino-N and CCS

Interpretation of relationships between amino-N and CCS need to be analysed with caution due to the complex interactions between plant physiology, the plants growing environment, harvesting and transport conditions and the factory's operating status that influence the final laboratory measured CCS of a rake of cane. To establish the extent of the relationship that exists at mill scale between amino-N and CCS a large data set needs to be analysed to ensure all factors that may be influencing CCS are represented. In any season between 15 000 and 25 000 rakes of cane may be processed through a mill. To produce a large enough data set amino-N needs to be measured universally, such as via NIR. All rakes are tested routinely for CCS.

From season 2000 a total of 617 rakes of cane had juice samples collected for NIR validation and equation calibration, comprising 331 rakes from Mulgrave Mill and 286 from Mossman Mill. These juice samples were analysed for amino-N via the laboratory ninhydrin reactive test. The NIR equation did not validate satisfactorily for amino-N. Consequently, for accuracy, NIR measured values of amino-N cannot be used to establish relationships between CCS and amino-N. Only those rakes analysed via the ninhydrin test are presented here.

Factors that influence CCS

Relationships between amino-N and CCS generated from a data set consisting of only 617 samples need to be considered with caution. While some relationships may trend in a real direction, conclusions can not be general due to the many factors that influence the final laboratory measured CCS value of a rake of cane.

There are many interacting factors that influence CCS. In the field these can be grouped into plant physiological factors and the growing conditions the plant experienced. The harvesting and transporting conditions experienced by the crop may also influence CCS, as well as factory operating conditions at the time of processing.

Plant physiological factors that may influence CCS include variety, crop class, age of crop at harvest and the composition of the 'cane' that is sent to the factory. The growing environment conditions that may influence CCS include the type of season (weather), soil type and structure, nutrition supply (particularly nitrogen) and water supply to the crop. Harvesting and transport conditions that differ in time (within the year and within the day), speed, machinery condition and age, and individual operator may influence CCS. The Mill the cane is sent to and factory efficiencies such as shredder operation may vary through time, and this may also influence CCS. These factors that potentially influence CCS may be intrinsically linked. For example a crop heavily supplied with nitrogen may be very 'leafy' and full of suckers, consequently extraneous matter in the harvested crop could be higher than average, which may affect the factories efficiency at extracting sugar in first expressed juice, where the measurement of CCS is taken.

It is because of the many factors that may be influencing CCS that a large data set is crucial when attempting to establish a relationship between CCS and amino-N. All potential variables within a population need to be represented in a relationship before the relationship can be described as robust. In the following graphs the data set has been 'drilled down' in an attempt to provide homogeneity of some of the factors which may have an influence on CCS.

Relationships between amino-N and CCS

The entire data set from 2000 of 617 samples, presented in figure 1, show no relationship between amino-N vs CCS due to the many factors influencing CCS. There is no suggestion in the following graphs any relationship that does exist is necessarily a linear one. Trend lines (linear regressions of datapoints) are only added to indicate the general shape of the data.

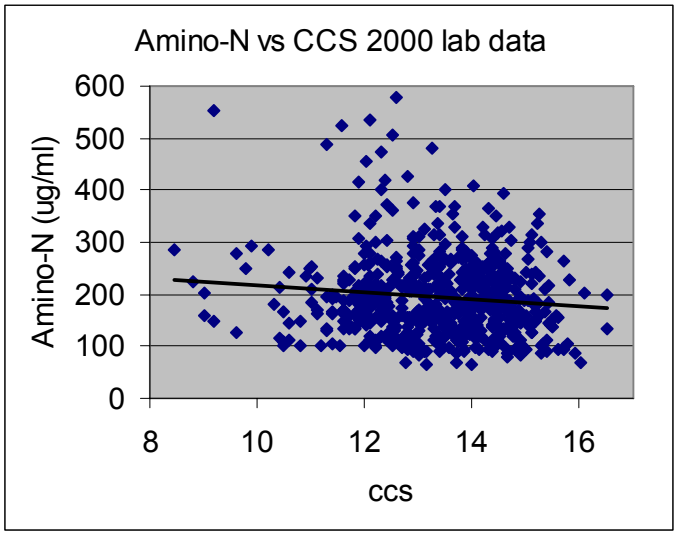


Figure 1: Relationship between amino-N and CCS from all samples taken for lab analysis during 2000 from Mossman and Mulgrave Mills.

The factory where a rake of cane is crushed is potentially one factor that influences CCS because of differing factory set up and efficiencies. The above data is divided into each respective mill in figure 2, however again due to many factors that influence CCS no relationship is apparent.

(a) (b)

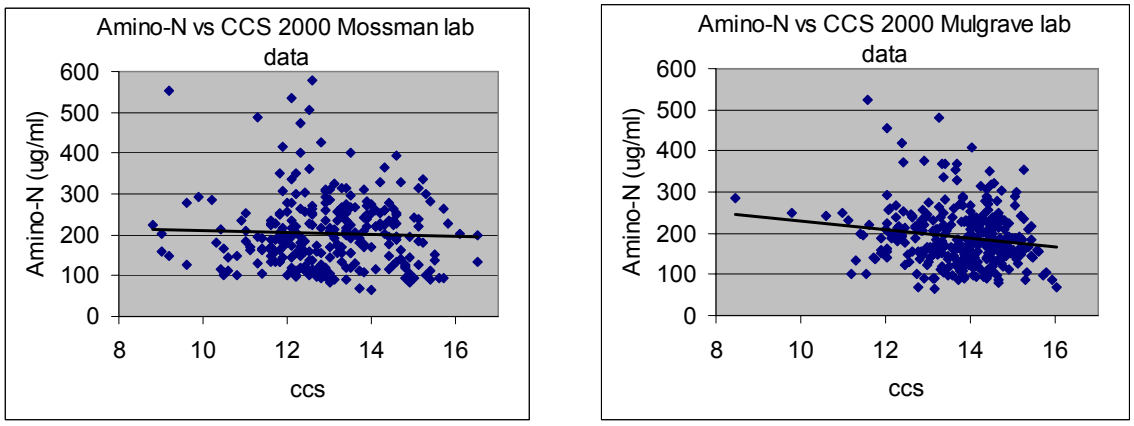


Figure 2: Relationship between amino-N and CCS from 2000 laboratory data from (a) Mossman Mill and (b) Mulgrave Mill.

Cane supplied to one mill can then be furthered sub divided into crop class (Mossman Mill data presented in figure 3). Crop class is one factor that is believed to influence CCS, particularly plant cane, crude relationships start to develop among our limited data set.

(b)

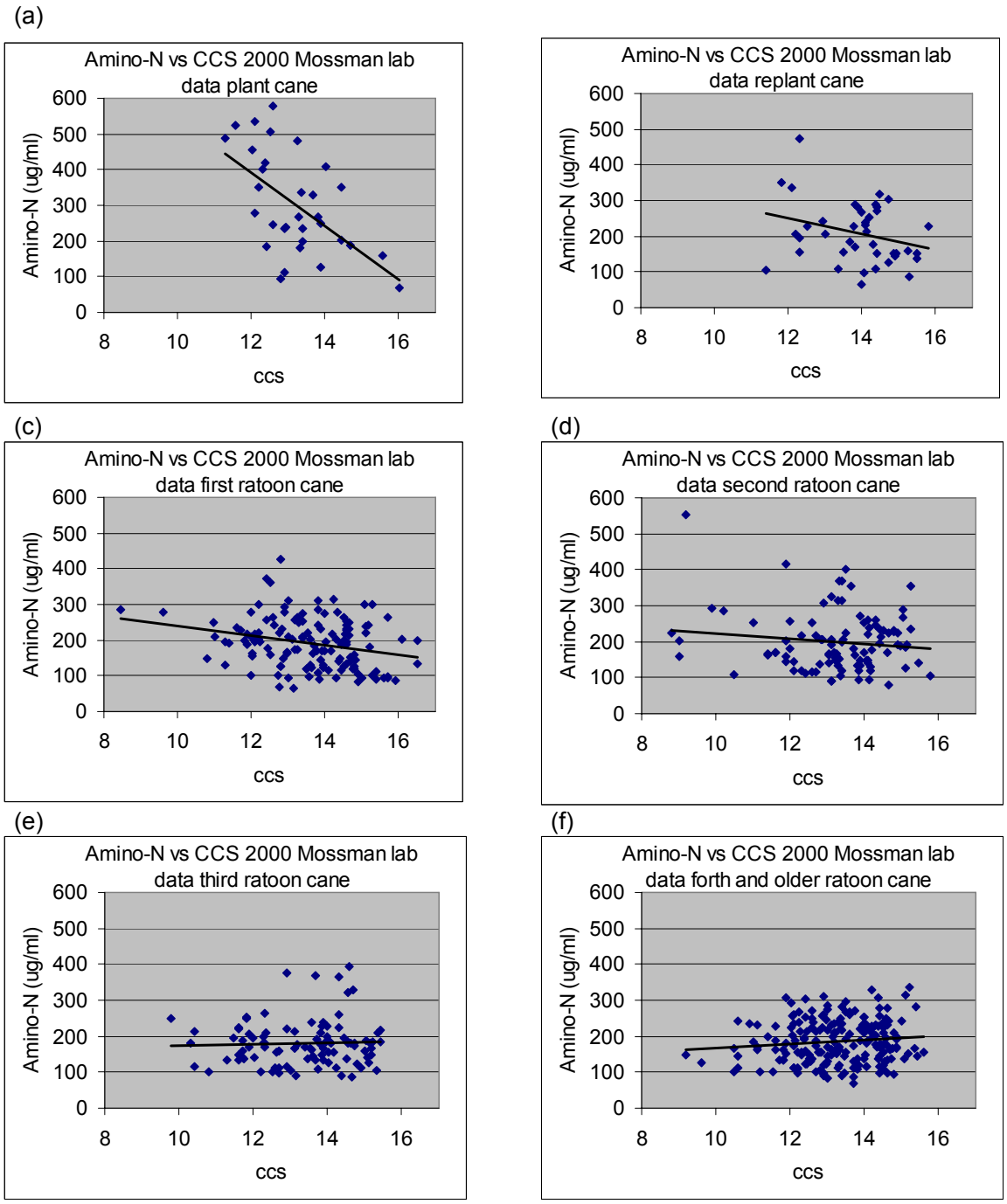


Figure 3: Relationships between amino-N and CCS at Mossman mill for (a) Plant cane, (b) Replant cane, (c) First ratoon, (d) Second ratoon, (e) Third ratoon, and (f) Forth and older ratoon.

The crude relationship shown above for plant cane may be influenced by the effect of plant cane generally having higher levels of amino-N than other classes of cane. This is probably due to the effect of the fallow, where nitrogen from organic sources in the soil is able to mineralise over the length of the fallow to inorganic, plant available, forms. Figure 4 confirms the average amino-N of plant cane as higher than the averages for other crop classes.

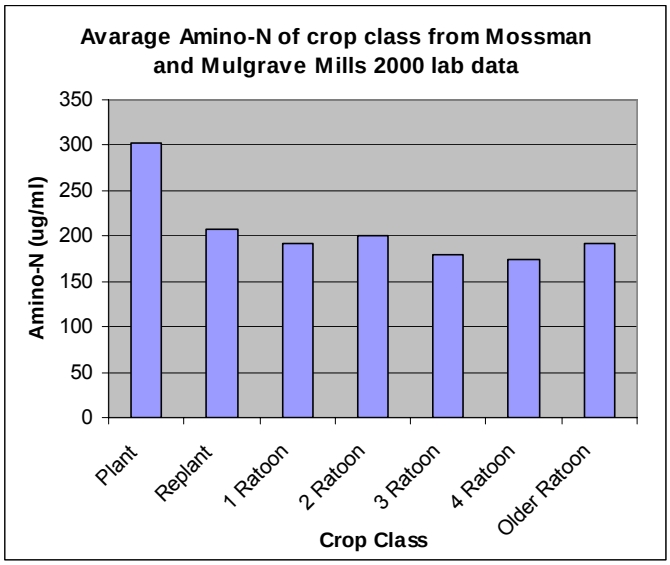


Figure 4: Average amino-N of individual crop classes from all Mossman and Mulgrave lab analysed amino-N data during season 2000.

When drilling down through this limited data set the number of data points becomes increasingly limited as the level of homogeneity rises. For example, figure 5 removes the variability of mill location, season, crop variety and crop class. It presents all the data we had for plant and 1st ratoon crops of the variety Q174 at Mulgrave Mill. Caution must be taken when viewing the relationship in figure 5 as there are still many factors that may influence CCS as noted previously.

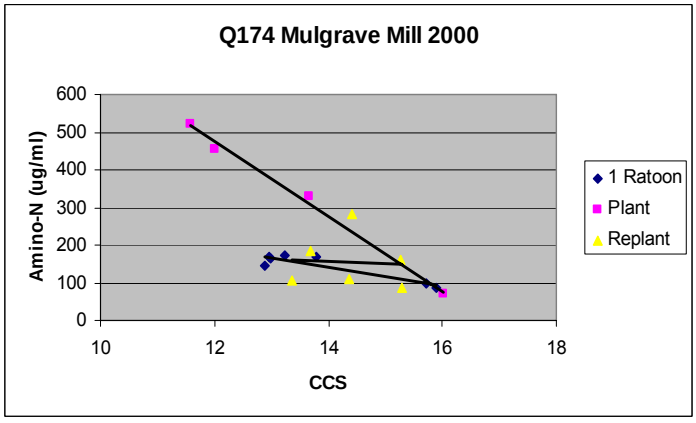
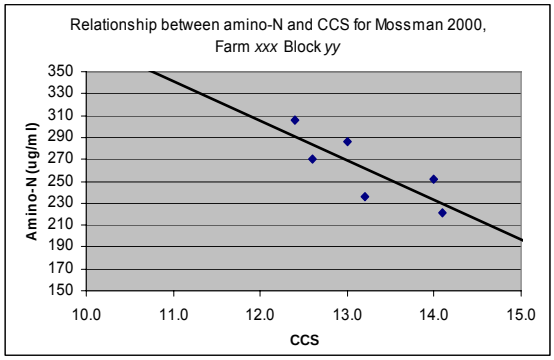


Figure 5: Relationships between amino-N and CCS for Q174 at Mulgrave Mill 2000. Individual trend lines for Plant cane, Replant cane and First ratoon.

The greatest level of homogeneity that could be extracted from this data set is when greater than two rakes were sampled from the single block. In this situation many of the factors that may influence CCS are the same for each data point. The caution that needs to be taken when interpreting these relationships is that not enough data points exist to be confident that the relationship that is being viewed is actually reflecting the relationship of the population. Figure 6 provides two examples of relationships between CCS and amino-N from single blocks from this data set.

(a)



(b)

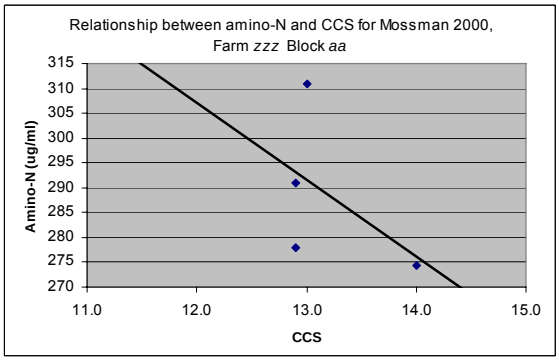


Figure 6: Relationships between CCS and amino-N from two individual blocks in Mossman Mill area sampled from 2000.

Summary

There are many factors that may influence the final CCS measured in a mill's juice lab: physiological condition of the crop, the growing conditions of the crop, harvesting and transport and mill operation and efficiencies. This great source of variability in CCS means small data sets interpretations such as the one presented here need to be analysed with caution. Relationships identified here may or may not be true reflections of the population.

Drilling down through this limited data set has shown a tendency for high levels of amino-N to be associated with decreased CCS. While the figures presented support this, there are many occasions where this data set does not show this relationship, or any relationship. When amino-N is high, CCS tends to be low. This does not amount to the inverse of 'when amino-N is low, CCS is high'.

With a robust NIR calibration equation for amino-N operating at the mill the number of data points available for data interpretation increases by many orders of magnitude. Having the capacity to capture amino-N values from all rakes of cane in a season will allow potential relationships between amino-N and CCS to be better identified. In terms of how the amino-N information might be ultimately used to address CCS issue, we can suggest the following logic.

CCS	Amino-N	Interpretation
CCS low	Amino-N low	Nitrogen supply unlikely to be the cause of low CCS
CCS low	Amino-N high	Excess nitrogen supply may be contributing to low CCS
CCS high	Amino-N low	Low CCS not an issue
CCS high	Amino-N high	Low CCS not an issue

Appendix 12: Validation of 5/00 global calibration equation for amino-N using juice samples collected during the 2000 crush.

Distribution plots of NIR and laboratory measured amino-N from Mulgrave and Mossman mills 2000 crush.

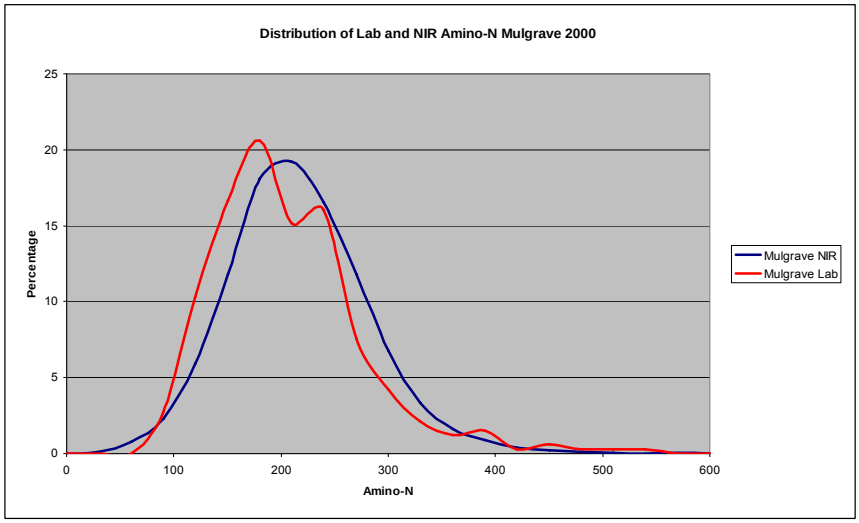


Figure 1: Distribution of amino-N for laboratory and NIR determined amino-N from Mulgrave mill during 2000 crush.

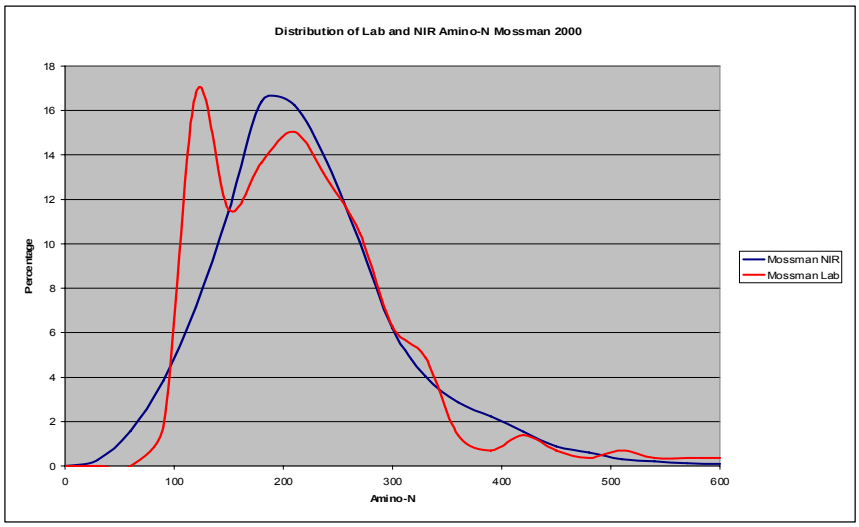


Figure 2: Distribution of amino-N for laboratory and NIR determined amino-N from Mossman mill during 2000 crush.

Figures 1 and 2 show the distribution of amino-N as measured by NIR is quite close to the actual distribution of amino-N as determined by ninhydrin reactive test is the laboratory. This indicates the

range of NIR's operation closely matches the range of amino-N observed. This is an improvement on the previous calibration equation for amino-N which could not measure amino-N values above approximately 350 ug/ml.

Deviation plots of distance NIR measured amino-N is away from laboratory measured amino-N for Mulgrave and Mossman mills during 2000.

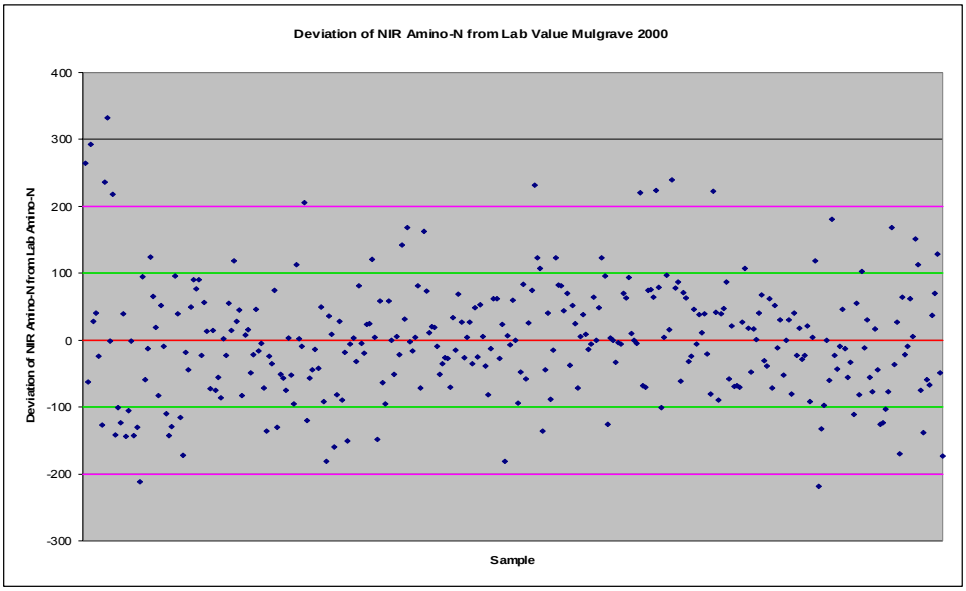


Figure 3: Deviation of NIR determined amino-N from laboratory measured amino-N for individual samples from Mulgrave mill 2000.

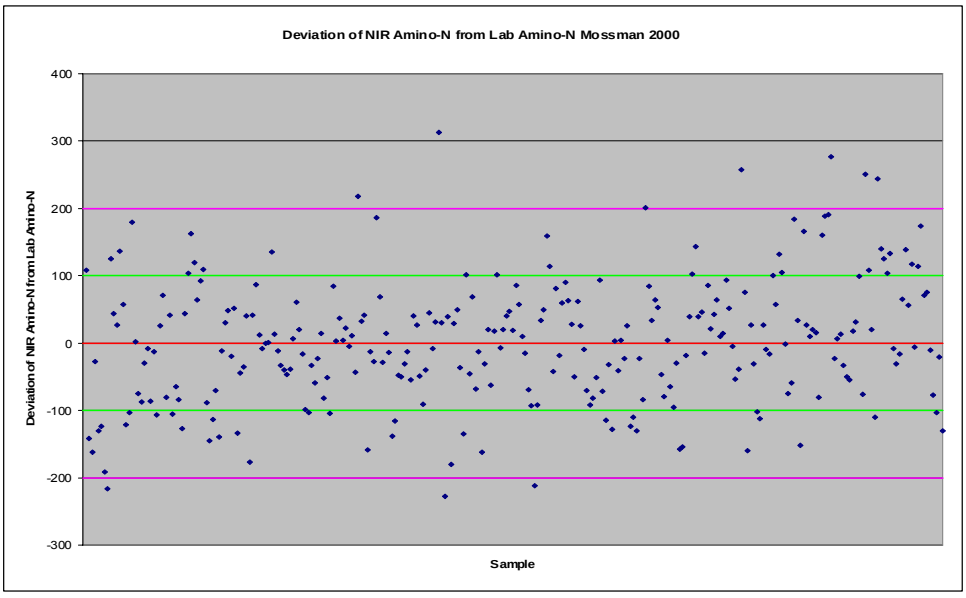


Figure 4: Deviation of NIR determined amino-N from laboratory measured amino-N for individual samples from Mossman mill 2000.

Figures 3 and 4 show the precision at which NIR measures amino-N is low. The error of NIR is up to 300 units, with the majority of errors in the ± 100 unit range. This level of error is too high for the project team to confidently communicate amino-N data with growers.

Validation plots of May 2000 global calibration equation for amino-N for Mossman and Mulgrave 2000 samples. Figures 5 and 6 are the validation plots of the May 2000 calibration equation using 2000 juice samples. The plots show there is little validation of the equation.

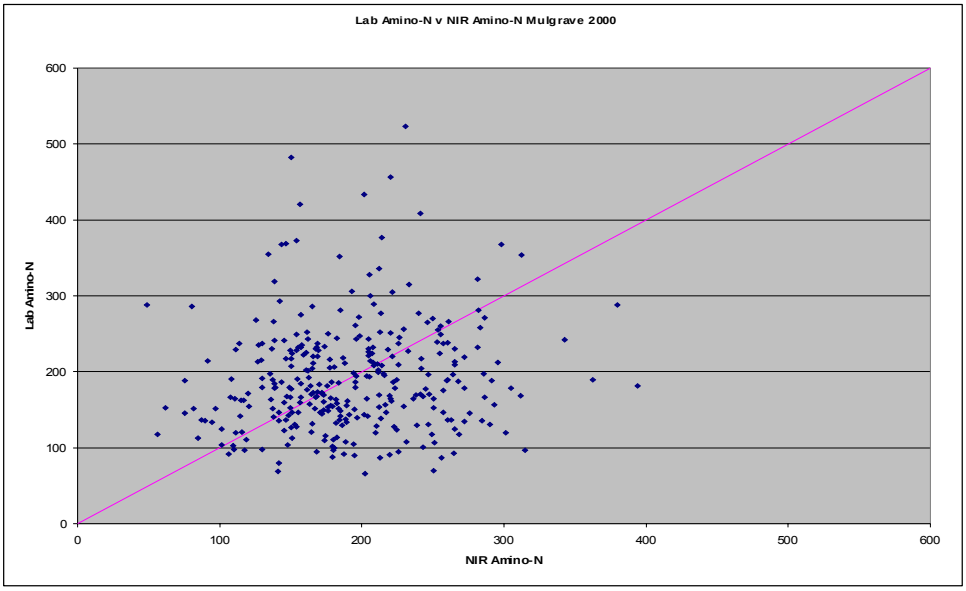


Figure 5: Validation plot of May 2000 global amino-N calibration equation, Mulgrave 2000.

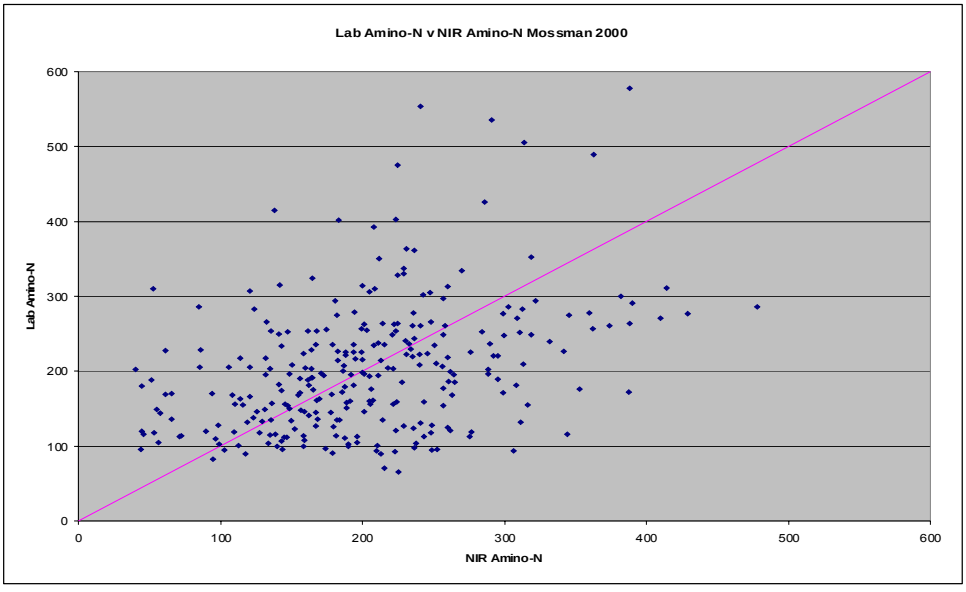


Figure 6: Validation plot of May 2000 global amino-N calibration equation, Mossman 2000.

Appendix 13: Validation analysis of 2001 global amino-N calibration equation.

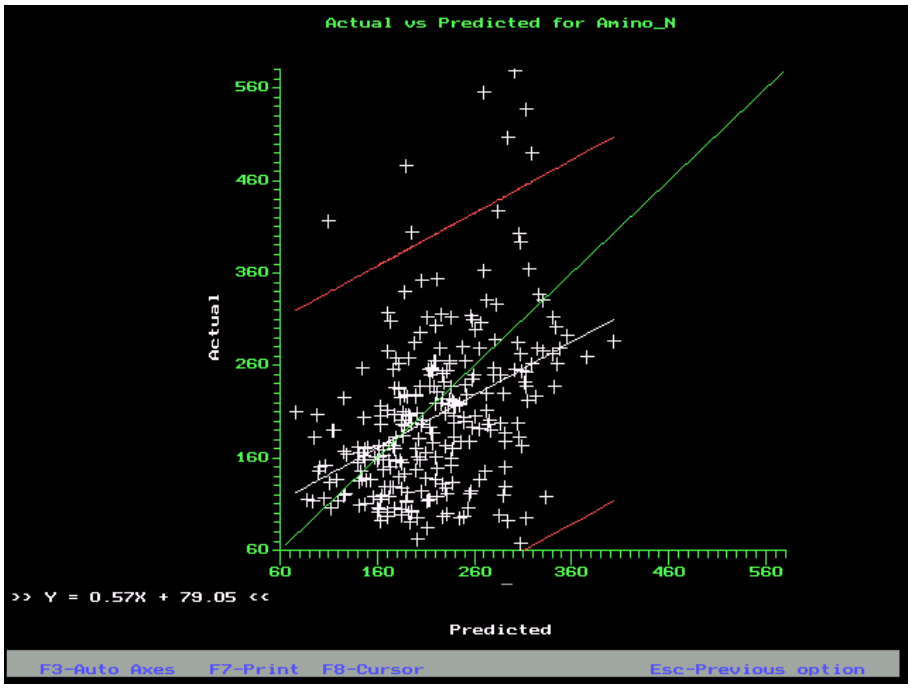


Figure 1: Validation plot of 2001 global calibration equation for amino-N using 2000 Mulgrave data. (NIR predicted amino-N is on the x axis, laboratory determined amino-N on the y).

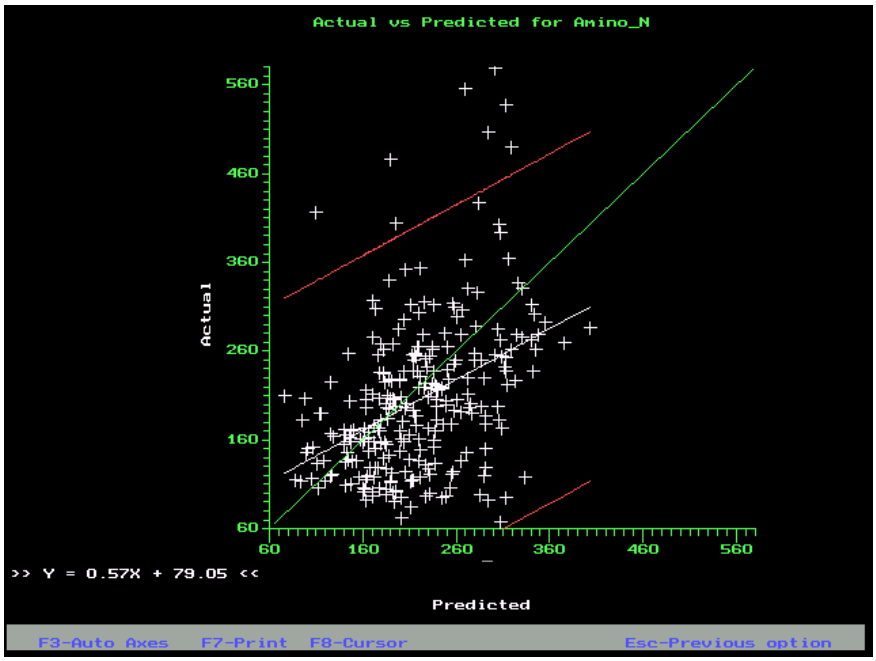


Figure 2: Validation plot of 2001 global calibration equation for amino-N using 2000 Mossman data. (NIR predicted amino-N is on the x axis, laboratory determined amino-N on the y).

Appendix 14: Report on how amino-N data has been presented to growers and their reactions to this data.

Tabular feedback to case study growers

At the completion of the 2000 crushing season project case study growers were presented their farms amino-N data. Growers were visited individually by the project researcher to present the data and explain the limitations of NIR in accurately measuring amino-N. Data presentation included both rake and block data. Individual rake amino-N data was presented in table form along with standard rake data. Block amino-N data was weighted for the contribution of individual rakes for presentation. Weighted block data was presented both as tables and colour coded farm maps.

Farm	Block	Sub-Block	Rake	Date	Variety	Class	Weight	CCS	Amino-N
####	1	1	882	3 Sept	Q158	OR	27.63	10.5	172
####	1	1	908	3 Sept	Q158	OR	63.45	10.1	197
####	1	1	938	3 Sept	Q158	OR	69.34	10.4	209
####	1	2	43	14 Aug	Q174	Replant	56.93	11.7	347
####	1	2	44	14 Aug	Q174	Replant	68.63	11.9	299
####	2	1	283	20 Sept	Q113	2R	64.32	11.4	262
####	2	1	285	20 Sept	Q113	2R	19.59	11.0	268

Table 1: Sample of individual rake amino-N reporting.

Farm	Block	Sub-Block	Variety	Class	Amino-N
####	1	1	Q158	OR	198
####	1	2	Q174	Replant	321
####	2	1	Q113	2R	263

Table 2: Sample of weighted block amino-N reporting.

The weighted block amino-N summary in Table 2 gives each block a single amino-N value regardless of the number of rakes that came from that block. Individual rake amino-N results are useful when interpreting amino-N data. Often one-on-one interpretation between the grower and the researcher is beneficial when anomalies exist on-farm that can not be detected when looking at the data alone.

Schematic feedback to case study growers

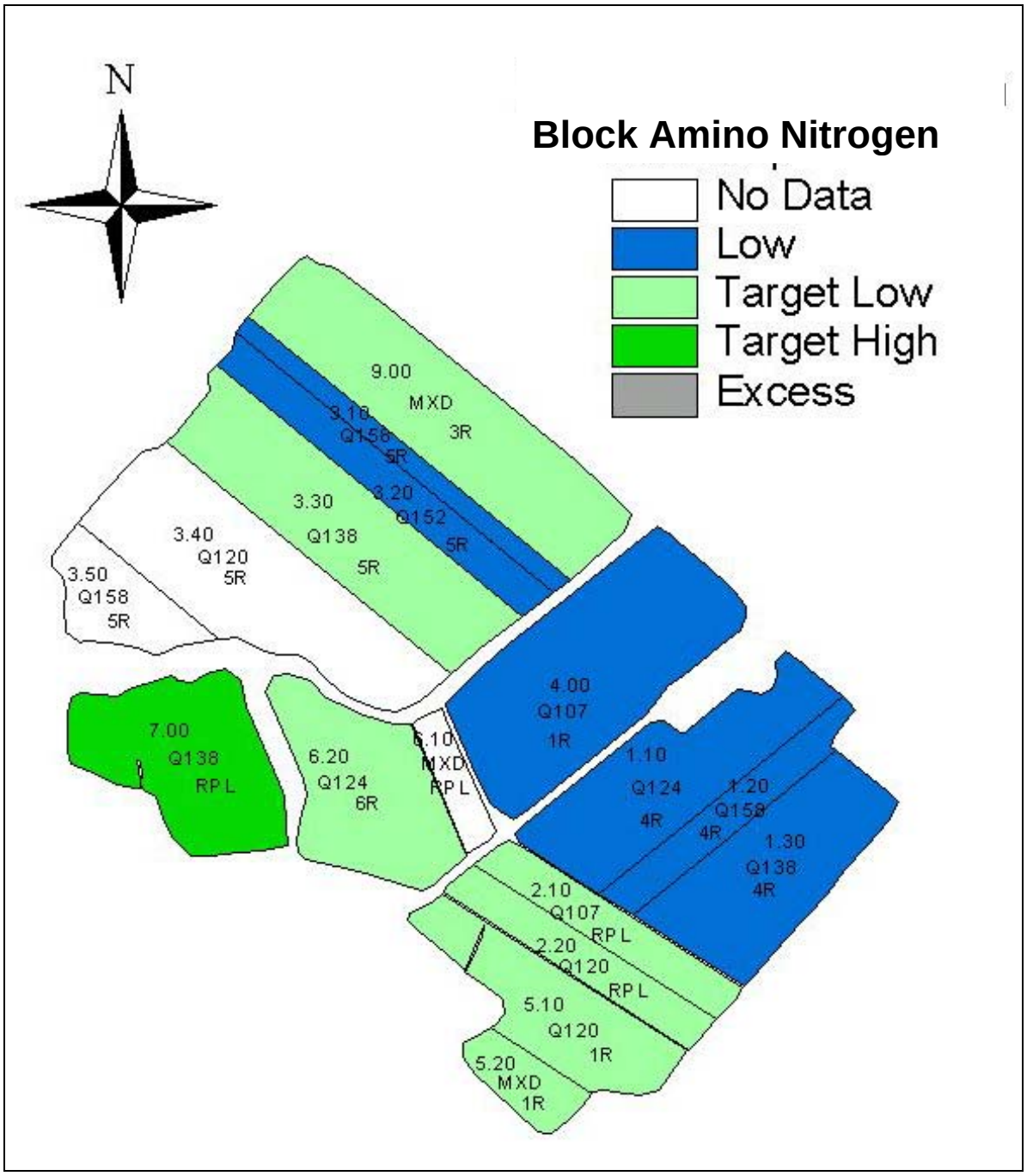


Figure 1:Sample farm map showing colour coded weighted block amino-N data.

Farm maps are a very useful tool for interpretation of data by growers. The above farm map presentation format was designed by collaboration between the researcher, mill staff and growers to be in a format amenable to interpretation by growers.

Guidelines for farmers using amino-N data

Guidelines for the appropriate use of amino-N data were produced earlier in the project life, and have been presented to SRDC in Milestone report 3, Appendix 9. These guidelines were distributed with rake and block information to growers at the completion of the 2000 crush. The guidelines are presented again here:

Guidelines for Cane Farmers on use of Amino Nitrogen Data

What is Amino Nitrogen?

Amino nitrogen is a measure of the nitrogen associated with amino acids in cane stems. Nitrogen is taken up and stored by sugarcane in the cane stem as amino nitrogen. When the growing plant requires stored nitrogen, it is transported from these amino acids to where the nitrogen is required.

Why measure Amino-N?

Amino nitrogen is measured in cane stems as supplied to the mill to provide a guide to the nitrogen status of the harvested crop. Measuring the level of nitrogen in amino acids in the cane juice (referred to as amino-N) provides this information.

Measuring amino-N is a very good method for picking up situations where nitrogen is in excess because sugarcane stores its excess nitrogen as amino-N.

How is Amino-N measured?

Amino-N is usually determined through a laboratory analysis of the cane juice called the *ninhydrin reactive test*. In a sugar mill, NIR is able to measure amino-N of the cane juice instead. Using NIR allows all rakes of cane delivered to the mill to be measured for amino-N.

What does Amino-N tell me?

Amino-N provides a guide as to the nitrogen status of the harvested crop. The Amino-N measurement is a reflection of the balance between nitrogen supplied to the crop, and that crops demand for nitrogen.

Amino nitrogen measures the balance between crop demand for nitrogen and nitrogen supply.

When amino-N is high, it is an indicator the nitrogen supplied to the crop was in excess of crop demand. When amino-N is low, it is an indicator the crops demand for nitrogen was not met sufficiently by the nitrogen supply.

The factors that influence crop demand for nitrogen and supply of nitrogen to a crop are the same factors that influence amino-N.

The Amino-N 'balance'

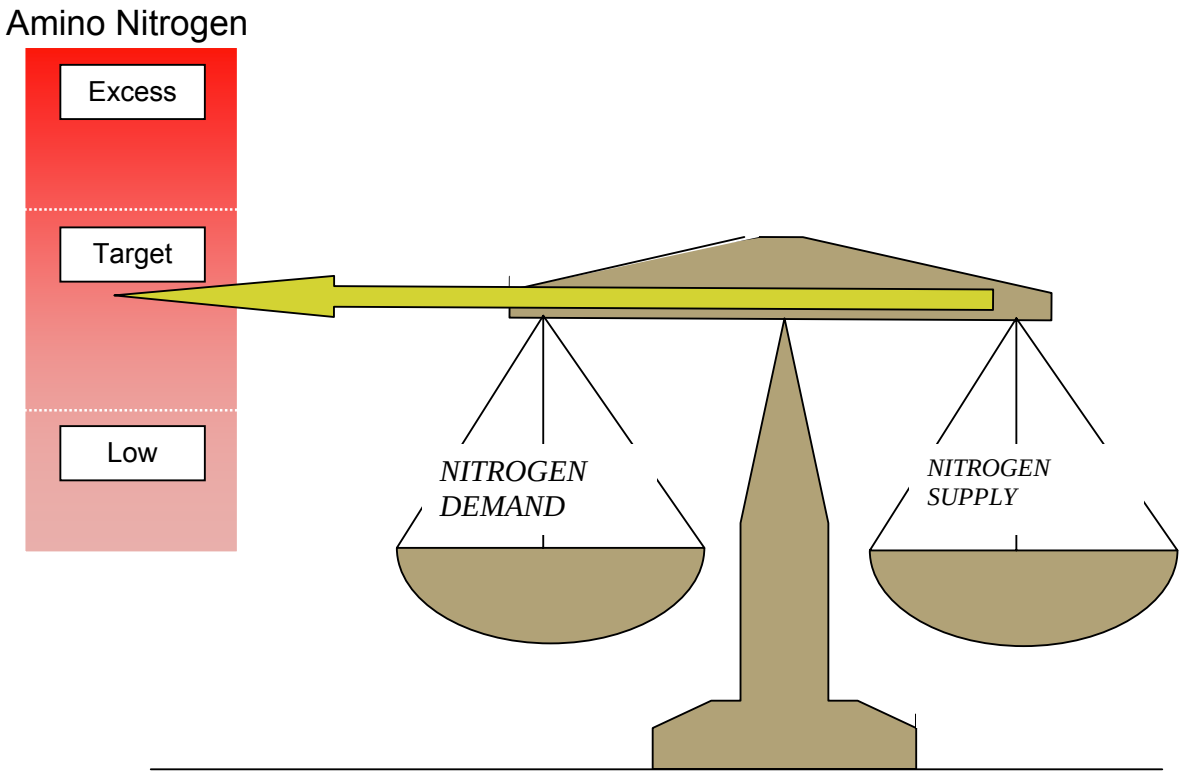
A balance can be used represent the amino-N measurement.

On one side of the balance is nitrogen supply. The greater the nitrogen supply, the further down the balance moves on this side.

On the other side is nitrogen demand. Crops with a large demand for nitrogen will cause this side of the balance to move downwards.

When crop demand and nitrogen supplied are in balance, amino-N is in the target range.

Target Range Amino Nitrogen

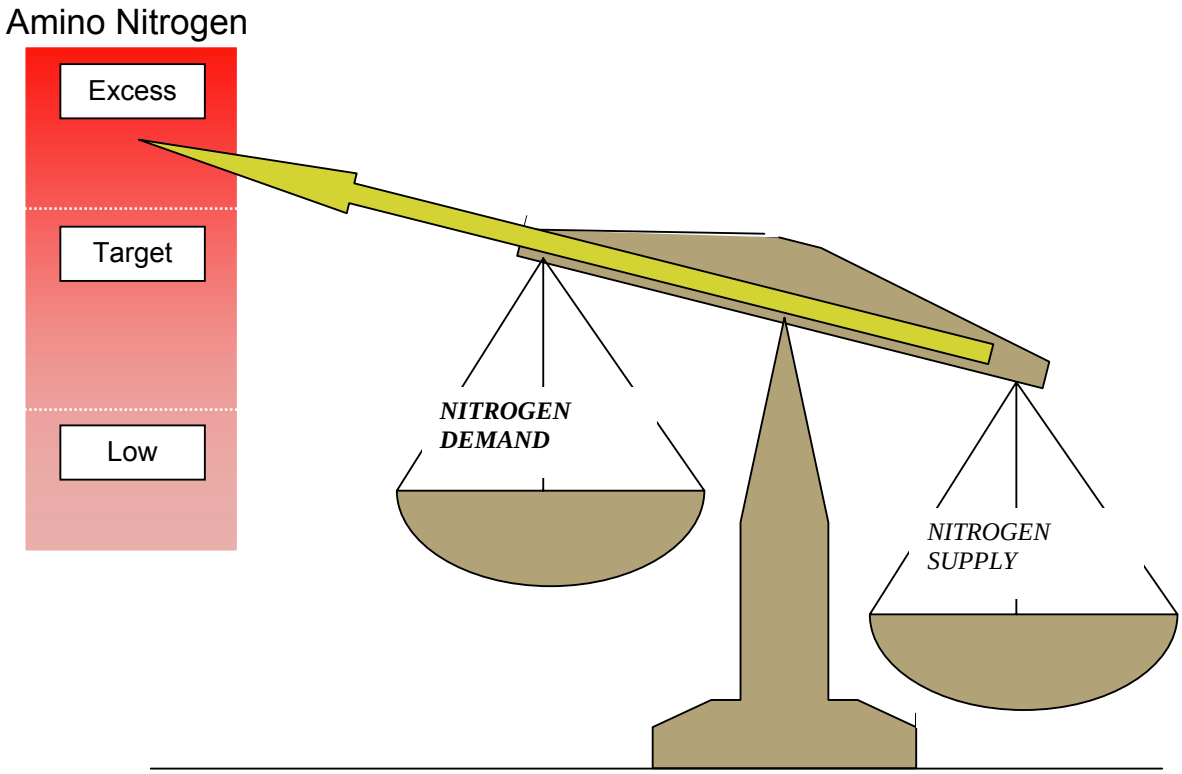


When the nitrogen demand of a crop and the nitrogen supplied to the crop are in balance, the amino nitrogen will be in the target range.

A small crop with a low nitrogen demand supplied with a low amount of nitrogen will be in balance. A large crop with a heavier nitrogen demand will also be in balance if it is supplied with more nitrogen.

Factors that influence **NITROGEN DEMAND**	Factors that influence **NITROGEN SUPPLY**
• Crop Size – weather – water logging – crop lodging – location – ratoon age – pest damage – disease incidence – other nutritional constraints • Possibly variety	• N fertiliser inputs (rates, forms, timing and methods) • Soil N sources (soil type and history) – mineral N residues – mineralisation • Mill wastes • Crop residues • Other minor N sources (rainfall, fixation) • N loss processes – leaching, denitrification, run off, volatilization

Excess Amino Nitrogen

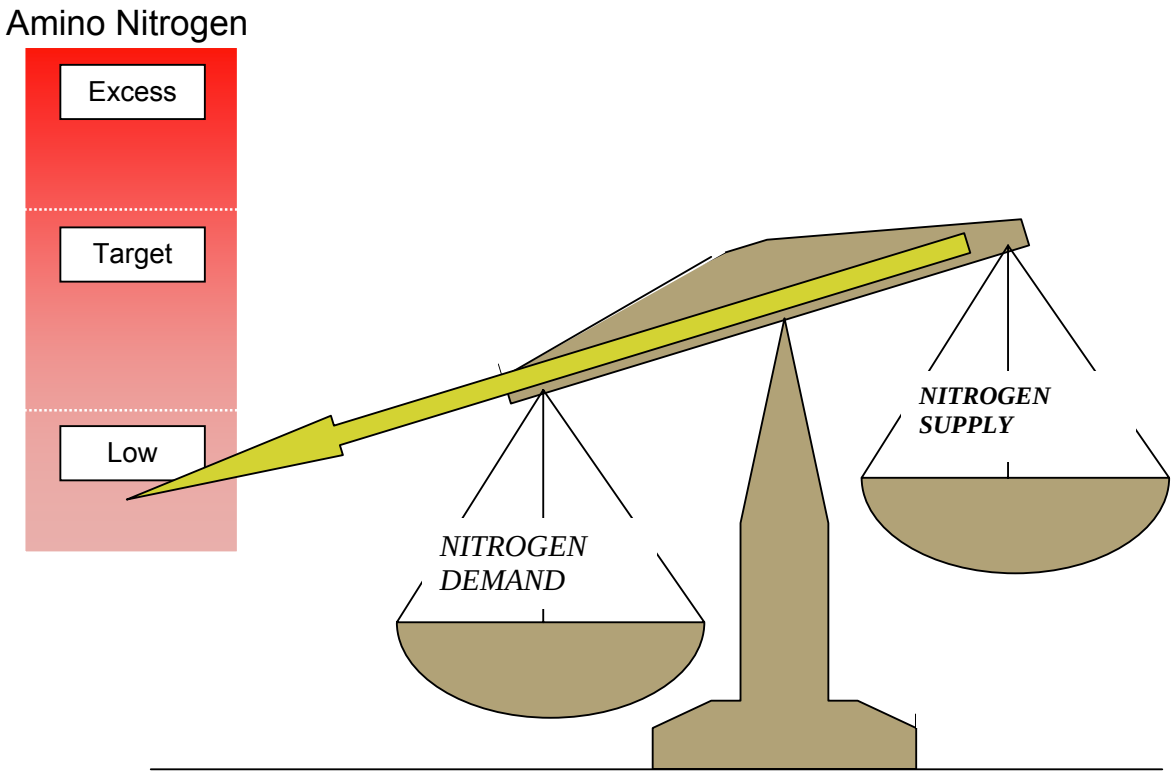


When amino-N is in the excess range it indicates nitrogen supply out-weighed nitrogen demand by the crop. This could result from growing a small crop, or over supply of nitrogen.

Excess amino nitrogen means nitrogen was wasted because there was more available than the crop needed. Excess amino-N suggests nitrogen may leave the farming system (because it is in excess), possibly contaminating surface or ground waters.

Factors that cause a light **NITROGEN DEMAND**	Factors that cause a heavy **NITROGEN SUPPLY**
• Any factor that causes small crop size: – Unfavourable weather – stool damage – water logging – crop lodging – very old ratoon – excessive pest damage – high disease incidence – other nutritional constraints • Loss of N fertiliser after application: – Leaching to ground water – Volatilisation to atmosphere – Run off to surface water – Denitrification to gases – Consumption by soil organic matter • Possible variety influences	• High N application for current crop • High residual N from high N application to previous crop • Mill mud or other wastes in addition to normal N fertiliser rate • Fallow ground prior to planting • Growing legumes prior to planting • Crop residues incorporated prior to planting • Soil N sources (influenced by soil type) – mineralisation – mineral N residues • Some minor N sources (rainfall, fixation)

Low Amino Nitrogen



Low amino-N indicates crop demand for nitrogen outweighed nitrogen supply.

Large crops have a higher demand for nitrogen than smaller crops. Low amino nitrogen will occur when not enough nitrogen is supplied to the crop, or when nitrogen is lost from the system before the crop is able to take nitrogen up.

Factors that cause a heavy **NITROGEN DEMAND**	Factors that cause a light **NITROGEN SUPPLY**
• Any factor that causes large crop size: – Favourable weather – Irrigation – Plant/Replant crops – Good stools – Adequate nutrition – Controlled pests and diseases • Possibly variety	• Low N fertiliser inputs • Applying unavailable forms of fertiliser • Incorrect fertiliser placement • N losses after application: – Leaching to ground water – Volatilisation to atmosphere – Run off to surface water – Denitrification to gases – Consumption by soil organic matter

Responses of growers to amino-N data

The responses of growers to amino-N information has generally been one of interest. It is unfortunate more reliable data cannot be produced at this stage of the project because growers are keen to fine tune their nitrogen management strategies with small scale on-farm strip trials, utilising amino-N measurements to monitor the effect of experimentation.

During 2000 a small number of case study growers initiated strip trials to try and fine tune individual blocks nitrogen management. Individual strip trials productivity data will be examined in conjunction with amino-N data to determine the effect of the experimentation. Details of the strip trials are:

- Q117 Replant and Q174 Plant cane fertilised with 120 kgN/ha, 149 kgN/ha and 164 kgN/ha.
- Strip trials of Dynamic Lifter at 5 bags/ha on Q174 Plant, Replant, 1R and 2R, H56 1R and 2R, Q135 3R. Dynamic Lifter applied on top of 175 kgN/ha.
- Split application of N in October and January to sandy, well drained block.
- Mill mud trials on top of normal N application
- Molasses application trials
- Effect of soybeans during fallow period on plant cane (and subsequent ratoons) vs conventional fallow.

Appendix 15: Report on the key steps necessary to successfully extend the N monitoring technique to other mill districts.

Introduction

Among the main aims of this research work are to implement the amino-N monitoring technique in an entire wet tropics mill area and to use amino-N information to address the low ccs issue. By following the steps described here it is possible to achieve both of these project objectives.

It is absolutely essential to this work that there is a system that can monitor amino-N levels of cane universally - every rake of cane needs to have an individual amino-N analysis. Single rake reporting forms part of amino-N feedback to growers. Rake information is then used to collate amino-N information into a block level reporting system. The amino-N for each rake from a block are weighted according to their contribution from that block and a single amino-N value assigned to the block. This weighted block amino-N information is the foundation of grower feedback mechanisms.

To successfully measure rake amino-N universally the NIR Cane Analysis System is utilised. At the present time the ability of NIR to accurately measure amino-N is limited. The researchers are taking many steps to rectify this situation including extensive HPLC (High Performance Liquid Chromatography) analysis and researching different sample preparations. For the purposes of this report it is going to be assumed NIR has the ability to successfully differentiate amino-N into low, target and excess groupings. These groupings form the basis of feedback to growers. A robust NIR calibration equation for amino-N is essential prior to any form of meaningful grower feedback.

The framework we are going to use here to describes the key steps necessary to successfully extend the N monitoring technique to a whole mill district. This framework starts with a situation analysis before identifying objectives and desired levels of client participation. Extension metaprocesses and microprocesses are then considered to perform the extension process. Metaprocesses are designed to achieve the stated objectives and microprocesses are the tools used to carry out the metaprocesses. A summary of the framework to be described here is:

1. Conduct a situation analysis
2. Set the objectives
3. Establish the learning principles
4. Describe the level of participation
5. Create the process (metaprocesses and microprocesses)
6. Develop the resources
7. Implement the process
8. Evaluate the process

Steps 1-4 for a generic wet tropics mill area will be described to give an indication of the issues the researchers are faced with and the learning principles to be used. Steps 5 and 6 are necessarily more descriptive explanations of the process that can be successfully used to implement the amino-N monitoring technique in an entire wet tropics mill area.

Conduct a situation analysis

A situation analysis provides the researcher with an overall picture of the environment they are about to operate in. A well conducted situation analysis provides the groundwork which underpins the rest of the process of program design.

Cane growers are generally ‘time poor’ during the sugarcane crushing season, with the majority of fertiliser application decisions being made during or immediately after the season. The new information made available to growers is fed back during the season, and

consequently any learning of this tool to help with nitrogen management decision making will occur during the season. Most growers are part of productivity groups or geographically based sub groups. Most mill areas are serviced by productivity officers associated with the mill and BSES extension officers who disseminate research information to growers, often via these small sub groups.

Currently world sugar prices are deeply depressed with the Australian raw sugar industry a price taker. The outlook for world prices is continued depression based largely on substantial production increases in Brazil. The previous 3-4 seasons have seen historically low sugarcane yields due to adverse growing conditions in the wet tropics. These two phenomena have contributed to the majority of growers reducing costs where practical including reduced spending on fertilisers. An increasing number of wet tropics growers are adopted the practice of growing soybeans in the fallow period prior to planting, contributing to the nitrogen content of the soil.

NIR Cane Analysis Systems are installed at four wet tropics mills (Mossman, Mulgrave, South Johnstone and Tully). This is the system used to universally monitor every rake of cane as it enters the mill. NIR is a cost effective tool for monitoring block information because all cane is delivered to the mill. NIR is utilised to monitor amino-N. The researchers have decided to assign amino-N 'values' to rake and block data. These values are low, target and excess. This three score system buffers some of the inherent variability associated with using a secondary measurement tool such as NIR and simplifies the feedback communication process.

Set the objectives

The process of setting extension goals should predispose the objectives to be measurable for evaluation. The researchers have chosen to set a hierarchal series of objectives. This hierarchy starts with extension inputs and resources, through a set of seven associated objectives, to a desired end results objective. High level objectives, such as desired end result, are often very difficult to evaluate, therefore lower level objectives are evaluated and supposition used to suppose these higher level objectives have been met.

The end result objective for this extension program is 'the maintenance of soil fertility and sustainable sugar productivity in the long term through improved fertiliser management'. The complete set of hierarchal objectives, in order from lowest to highest, are:

- Extension input of raw and weighed rake and block amino-N data presented in grower friendly format
- Activities include workshops to introduce growers to the conceptual basis of amino nitrogen and appropriate responses to the information
- The people involved include growers, mill staff, the CSIRO researcher, BSES extension staff and mill area productivity officers. Linkages with these people will help ensure a clarity of the message and maximise opportunities to get project output to growers
- Desired grower reactions include confidence the data is providing information of specific value to their blocks and farms and use of the amino-N tool to guide nitrogen management for optimal ccs on farm
- Desired changes in growers include knowledge of the amino-N concept, trust of amino-N data, skills in using amino-N data to guide nitrogen management on farm and aspirations to manage their nitrogen sustainably
- The desired change in grower practice is the more efficient use of nitrogen fertilisers to raise sugar industry profitability and minimise negative environmental impact from losses

- The end result objective is the maintenance of soil fertility and sustainable sugar productivity in the long term through improved fertiliser management

Establish the learning principles

It is important to recognise a set of adult learning principles to be used during an extension campaign to give all growers the opportunity to learn. The adult learning principles in use here are: help learners learn what they want to learn, recognise and value learners experiences, create a safe learning environment, involve learners in their learning, recognise learners are adults, encourage learners to act, encourage learners to reflect, encourage learners to draw conclusions, encourage learners to plan, help learners see if their learning has been successful.

Describe the level of participation

Participation by growers in this extension effort is essential to achieve the project goals, particularly the forming of groups to meet the above predetermined objectives. BSES extension staff and mill productivity officers will also be encouraged to participate to maximise extension opportunities and ensure clarity of the message growers are receiving.

Participation by growers will initially be via a workshop where the amino-N concept is explained and appropriate use of amino-N data on farm discussed. Participatory action research approaches will be used is designing guidelines for the appropriate use of amino-N data on farm specific to this mill area.

Create the process (metaprocesses and microprocesses)

Metaprocesses are broad topic areas to be conducted during the course of the extension campaign and are necessarily designated first. These metaprocesses are designed to achieve the project objectives. Microprocesses are specific strategies assigned to each metaprocess, and are the details used to ensure each metaprocess is achieved.

Metaprocess: Establish contact with people involved

Within a mill area where it is desired to extend the amino-N monitoring technique it is important that all stakeholders are advised about the process and given the opportunity for input to the project. Mill management should be contacted initially and asked for use of amino-N data collected from the NIR system as well as mapping and productivity information. Mill management should also be given a brief overview of the project objectives.

Within a mill there is usually one person designated responsible for the collation of NIR data and the maintenance of the NIR system. This person, usually the production superintendent or senior chemist, should be identified and contacted as NIR output is essential to grower feedback. The mill IT officer should concurrently be contacted to help with accessing and collating appropriate data.

Mill productivity officers and BSES extension officers operating in the mill area should be contacted as these people provide the best links with growers. Keeping these officers informed of the projects objectives and progress also helps ensure the message going to growers about amino-N is consistent. Productivity and BSES extension staff are able to give advice on the best methods and opportunities for extending the projects outputs to growers.

Grower representative bodies CANEGROWERS and Australian Cane Farmers Association should be contacted within the mill area and informed of the amino-N projects broad strategy and objectives.

Metaprocess: Design systems which have an output of amino-N data in a form acquiescent for grower interaction

Grower feedback mechanisms have been established through participatory processes with case study grower groups in the Mossman and Mulgrave mill areas. Feedback of amino-N information to growers occurs in two separate but corresponding ways. During the season

growers receive individual rake amino-N data along with their traditional cane receival feedback. At the end of the season, when growers receive a productivity report for their whole farm, amino-N data is fed back for the entire season also. In season reporting consists of each rake assigned an amino-N 'score' of being either low, target range, or excess. End of season reporting includes a thematic colour coded map with weighted block amino-N scores again divided into low, target range, or excess amino-N. End of season reports also contain a summary of rake data for each block assessed against productivity data for that block.

The aim for amino-N feedback to growers in a new mill area is to utilise current grower feedback mechanisms in use by the mill. Rake productivity data (such as ccs, fibre, bin weights) is generally fed back to growers either daily, weekly or fortnightly. Ideally amino-N data can be fed back to growers in the same process. Mill IT staff should be consulted prior to the season to allow this process to be set up. Productivity officers usually complete end of season productivity reporting within two weeks of a season finishing. Amino-N data collated for the season should be presented in thematic maps and tables with productivity data. To achieve this mill IT staff should be consulted to allow amino-N data to be presented in this way.

During the implementation of amino-N feedback to all growers in a mill area database systems linking NIR, rake data, mapping and productivity reports need to be established. Amino-N feedback to growers needs to be able to occur automatically in subsequent season without input from the researcher.

Guidelines on how growers should interpret amino-N data and how the data can be used to guide nitrogen management for optimal ccs on farm should accompany initial in season and end of season feedback. These guidelines will be discussed in more detail below.

Metaprocess: Describe the conceptual basis of amino-N monitoring

In consultation with mill productivity and BSES extension officers opportunities should be identified where the researchers can work with small grower groups. Within these small groups the amino-N monitoring program can be fully explained and the conceptual basis of amino-N described. Project CTA029 identified much of the scientific principles underlying the amino-N monitoring technique. These principles should be explained to growers with examples of each principle used.

Essentially the concept of monitoring amino-N is that amino-N is based on the nitrogen content of cane at harvest, measured at the mill, related back to a specific block, and used to adjust nitrogen management on that particular block in subsequent crops. The reasoning behind the monitoring is there is a large downside risk of inadequate nitrogen, and a marginal cost of 'insurance' (additional just to make sure) nitrogen application. Because the optimum nitrogen level has been unable to be accurately determined, uncertainty has generated risk avoidance, leading to excess nitrogen applications. Monitoring amino-N has the ability to determine the optimum levels of nitrogen, increasing the growers ability to manage their crop.

Important aspects of the concept that should be explained are

- Background to why this research work has been conducted (nitrogen fertiliser rates have risen faster than cane yields, there is evidence of excess nitrogen fertiliser applications and excess nitrogen can damage the environment)
- Consequences of excess nitrogen (increased cost to growers for fertiliser, reduced ccs, nitrogen losses to the environment, detrimental impact of excess nitrogen on the soil – acidification)
- Strengths of the amino-N monitoring concept (because monitored at harvest it integrates all factors contributing to the supply and demand of nitrogen supply, good technique for identifying nitrogen excess situations, universal sampling of all rakes in

a mill district, measurement is used as a basis for subsequent nitrogen management, amino-N is more responsive than leaf nitrogen to changes in nitrogen content)

- Broad monitoring concept (based on the nitrogen content of cane at harvest, measured at the mill, related back to a specific block, and used to adjust nitrogen management on that particular block in subsequent crops)
- Factors that can affect amino-N (nitrogen fertiliser application, water stress, possibly variety, components of cane supply)
- Factors that influence the amino-N of cane supply (crop nitrogen demand and nitrogen supply to the crop)
- Relationships between crop nitrogen status, as measured by amino-N, and ccs at the block level (how increased nitrogen can be detrimental to ccs)

These concepts should be supported with data from the case studies in Mossman and Mulgrave to illustrate actual examples of the points being raised. Because people learn in different ways it is important that these concepts are presented to growers in a number of ways (visual presentation, verbal explanation, take home notes) and opportunities to ask questions are given (both in group situation and after to either researcher or productivity or BSES officers). By catering for each of the learning methods there is an increased probability more growers will adopt the amino-N monitoring technique.

Metaprocess: Establish familiarity and confidence with feedback mechanisms and guidelines for appropriate use of amino-N data among growers, productivity officers and BSES extension staff

Feedback of information to growers needs to be in a form in which the growers can understand and interpret. Productivity and BSES extension officers should also have the ability to interpret amino-N data confidently, and should be trained by the researchers. During the workshop session to describe the conceptual basis of amino-N monitoring the feedback mechanisms should also be described. Generic examples of in season and end of season feedback should be shown and discussed. These examples need to be in the format that will be used for grower feedback in this mill area.

In season feedback consists of each rake of cane delivered to the mill being assigned an amino-N value. The value is either low, target or excess, depending on the amino-N as measured via NIR. These values should be printed on the rake information feedback mills currently provide to growers during the season. This way once the mechanism to put amino-N information on the feedback forms has been set up, it should occur automatically for every rake through the entire season.

End of season feedback is collated rake data synthesised into block data. Rake amino-N scores are weighted depending on their contribution from the block and a single value assigned to the block, again either low, target or excess. These values are then presented on a colour coded thematic map of each farm. Most mill areas provide map based feedback of productivity data at the end of each season. Amino-N data feedback should accompany this current end of season feedback. A database system which collates and synthesises amino-N data into the form described here for end of season feedback should be established for each mill area.

Established guidelines for use of amino-N data should also be distributed in a ‘draft’ form. The guidelines identified by Mossman and Mulgrave case study growers should be used. Participatory action research approaches should then be used to develop an appropriate set of guidelines specific to the mill area. Using these approaches allows specific issues associated with the mill area to be included and increases the ownership growers have of the guidelines.

The present guidelines describe amino-N, present reasons for monitoring amino-N, describe how it is measured and illustrates how amino-N data can be used to optimise ccs on farm. A useful method for describing what amino-N is actually measuring and how amino-N data can be interpreted is to use a balance. Mossman and Mulgrave grower case study groups associate with amino-N measuring the balance between crop demand for nitrogen and nitrogen supply. Factors that influence nitrogen demand (crop size - weather, water logging, lodging, location, age, class, pest damage, disease incidence, nutritional constraints, and possibly variety) and factors that influence nitrogen supply (fertiliser inputs, soil sources, mill wastes, crop residues, nitrogen loss processes) are listed. If the nitrogen demand increases, or the nitrogen supply decreases, or a combination of these two, then amino-N will decrease. If the nitrogen demand is low, or the nitrogen supply is large, or a combination of these two, then amino-N will increase.

These guidelines should be presented to growers in the initial workshop session as a draft format for appropriate use of the data. Participatory approaches should then be used to come up with alternate guidelines that may be specific or more relevant to the mill area. This should then be the guidelines used when feedback of amino-N data occurs in the initial season.

Metaprocess: Provide real time feedback and end of season feedback of amino-N data to an entire mill area

Once the season starts the processes should be in place to allow in season amino-N feedback to all growers. This feedback should occur with the current rake information feedback conducted by the mill. Database systems should be in place to allow amino-N data to be collated and synthesised in to end of season reports, including thematic mapping. This form of reporting should also occur concurrently with current mill reporting procedures. During the initial season it is important for guidelines on how to use amino-N data to better manage nitrogen for optimal ccs on farm to be sent to growers along with amino-N feedback.

Develop the resources

After studying the microprocesses planned for implementation it is apparent there are a number of physical resources that will be needed. These resources should be developed as early as practical in the extension program. The resources needed for this program that can be prepared prior to implementation are:

- Letter to mill manager asking for permission to utilise NIR amino-N data and link with productivity data where appropriate
- Case study group developed in season feedback format examples for showing to growers
- Case study group developed end of season feedback format examples
- Case study growers guidelines on appropriate use of amino-N information

Letter to mill managers seeking permission for access to data

Dear Mill manager,

You are probably aware of the new project focussing on enhancing on-farm nitrogen management via interpretation of amino-N levels in cane delivered to the mill as measured by NIR. The project is coordinated by CSIRO, with input from BSES and Sugar North and the support of SRDC's CP2002 initiative on sugar industry productivity in the wet tropics. I am writing to you to ask for appropriate permission to access data from your mill to allow this project to produce meaningful outcomes.

By utilising NIR, this project is aiming to interpret cane animo-N levels in consultation with cane farmers to arrive at a better understanding of nitrogen issues at a block level on individual farms. This interpretation can then be fed into fertiliser management practices for ensuing sugarcane crops. This should result in an enhanced level of on-farm nitrogen management, benefiting both the cane grower and miller. The research will also explore the links between nitrogen management and CCS achieved.

I am aware of data access concerns and confidentiality issues that are associated with the release of block data to third parties. I can assure you that the sole purpose of this project is to enhance on-farm nitrogen management practises, with data interpretation to occur consultatively with cane farmers. The data required includes rake and block data (date, farm/block origin, yield, area, variety, CCS, crop class, fibre) along with matching NIR results for amino-N levels. I will be communicating project objectives with grower representative bodies and there will be full reporting of progress within the project to the mills, grower groups and SRDC. Care will be taken in any reporting not to present data in any way that could be dis-advantageous to individual growers.

I am therefore requesting from *xyz* mill permission to access data during the coming crushing season. Should you have any questions or concerns regarding any aspect of this project or data access, please do not hesitate to contact me on 4030 4148. I am also willing to visit *xyz* mill to discuss any issues further with you. I look forward to receiving a reply from *xyz* mill regarding this issue.

Yours Sincerely

Tony Webster

RESEARCH AGRONOMIST

cc. Dr Brian Keating, CSIRO Sustainable Ecosystems

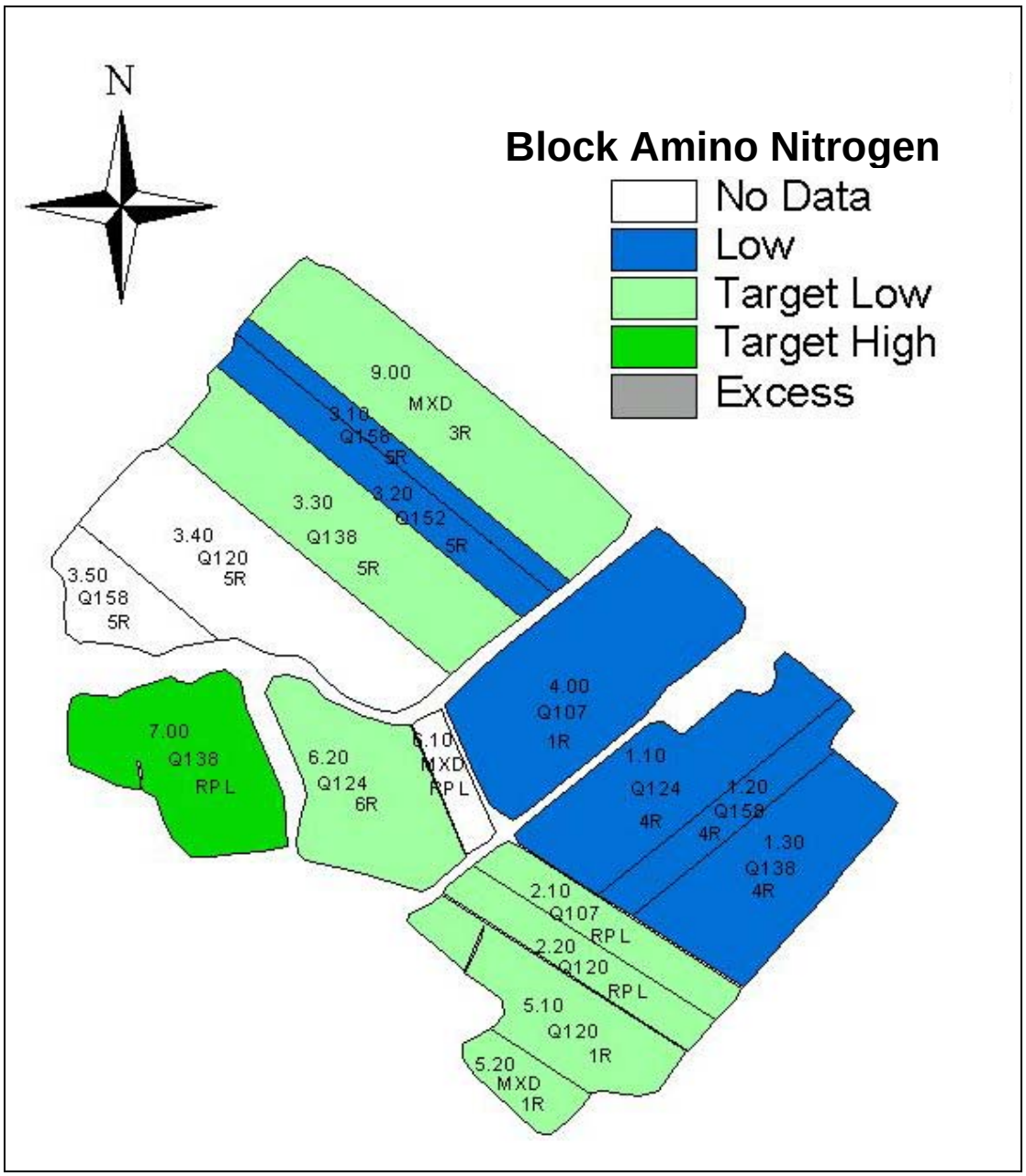
Mr Steve Staunton, BSES Meringa

Sample in season amino-N data feedback

Farm	Block	Sub-Block	Rake	Date	Variety	Class	Weight	CCS	Amino-N
####	1	1	43	3 Sept	Q158	OR	27.63	10.5	Target
####	1	1	44	3 Sept	Q158	OR	63.45	10.1	Target
####	1	1	283	3 Sept	Q158	OR	69.34	10.4	Target
####	11	2	285	5 Sept	Q174	Replant	56.93	11.7	Excess
####	11	2	882	5 Sept	Q174	Replant	68.63	11.9	Excess
####	2	1	908	5 Sept	Q113	2R	64.32	11.4	Excess
####	2	1	938	5 Sept	Q113	2R	19.59	11.0	Excess

Sample end of season amino-N data feedback

Farm	Block	Sub-Block	Variety	Class	CCS	Amino-N
####	1	1	Q124	4R	12.4	Low
####	1	2	Q158	4R	13.8	Low
####	2	1	Q107	RP	14	Target



Guidelines for Cane Farmers on use of Amino Nitrogen Data

What is Amino Nitrogen?

Amino nitrogen is a measure of the nitrogen associated with amino acids in cane stems. Nitrogen is taken up and stored by sugarcane in the cane stem as amino nitrogen. When the growing plant requires stored nitrogen, it is transported from these amino acids to where the nitrogen is required.

Why measure Amino-N?

Amino nitrogen is measured in cane stems as supplied to the mill to provide a guide to the nitrogen status of the harvested crop. Measuring the level of nitrogen in amino acids in the cane juice (referred to as amino-N) provides this information.

Measuring amino-N is a very good method for picking up situations where nitrogen is in excess because sugarcane stores its excess nitrogen as amino-N.

How is Amino-N measured?

Amino-N is usually determined through a laboratory analysis of the cane juice called the *ninhydrin reactive test*. In a sugar mill, NIR is able to measure amino-N of the cane juice instead. Using NIR allows all rakes of cane delivered to the mill to be measured for amino-N.

What does Amino-N tell me?

Amino-N provides a guide as to the nitrogen status of the harvested crop. The Amino-N measurement is a reflection of the balance between nitrogen supplied to the crop, and that crops demand for nitrogen.

Amino nitrogen measures the balance between crop demand for nitrogen and nitrogen supply.

When amino-N is high, it is an indicator the nitrogen supplied to the crop was in excess of crop demand. When amino-N is low, it is an indicator the crops demand for nitrogen was not met sufficiently by the nitrogen supply.

The factors that influence crop demand for nitrogen and supply of nitrogen to a crop are the same factors that influence amino-N.

The Amino-N 'balance'

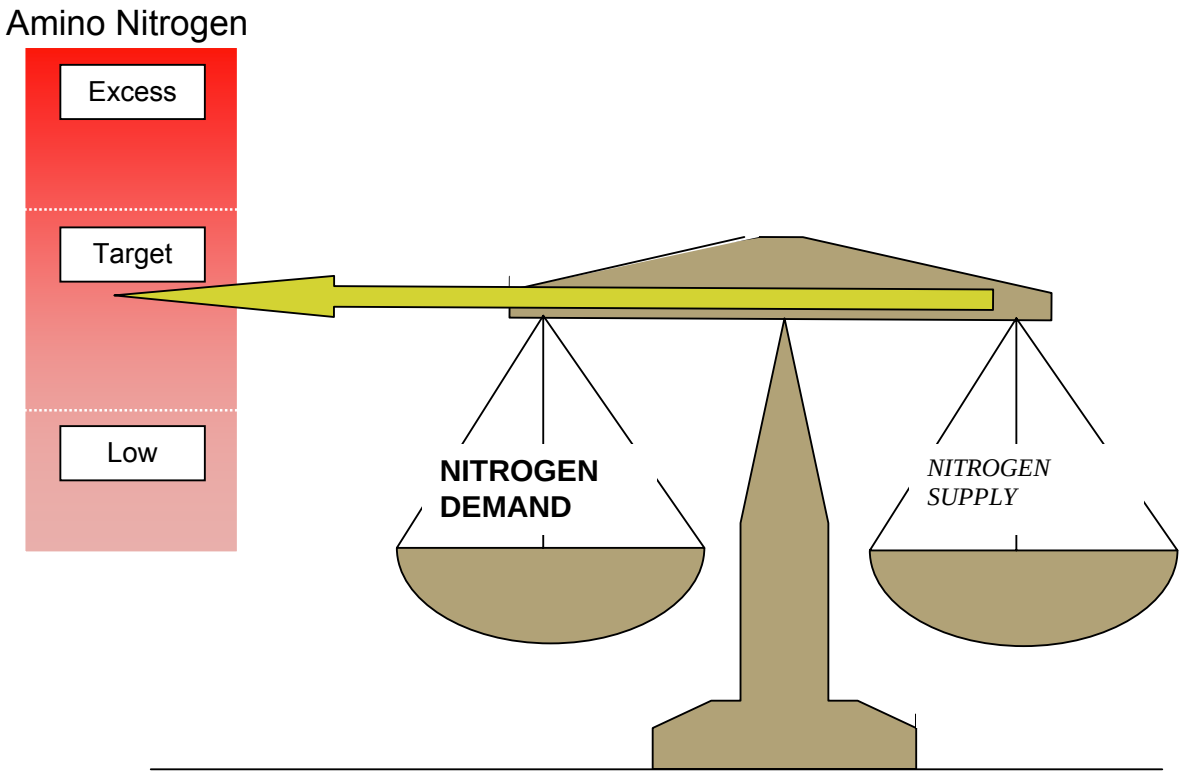
A balance can be used represent the amino-N measurement.

On one side of the balance is nitrogen supply. The greater the nitrogen supply, the further down the balance moves on this side.

On the other side is nitrogen demand. Crops with a large demand for nitrogen will cause this side of the balance to move downwards.

When crop demand and nitrogen supplied are in balance, amino-N is in the target range.

Target Range Amino Nitrogen

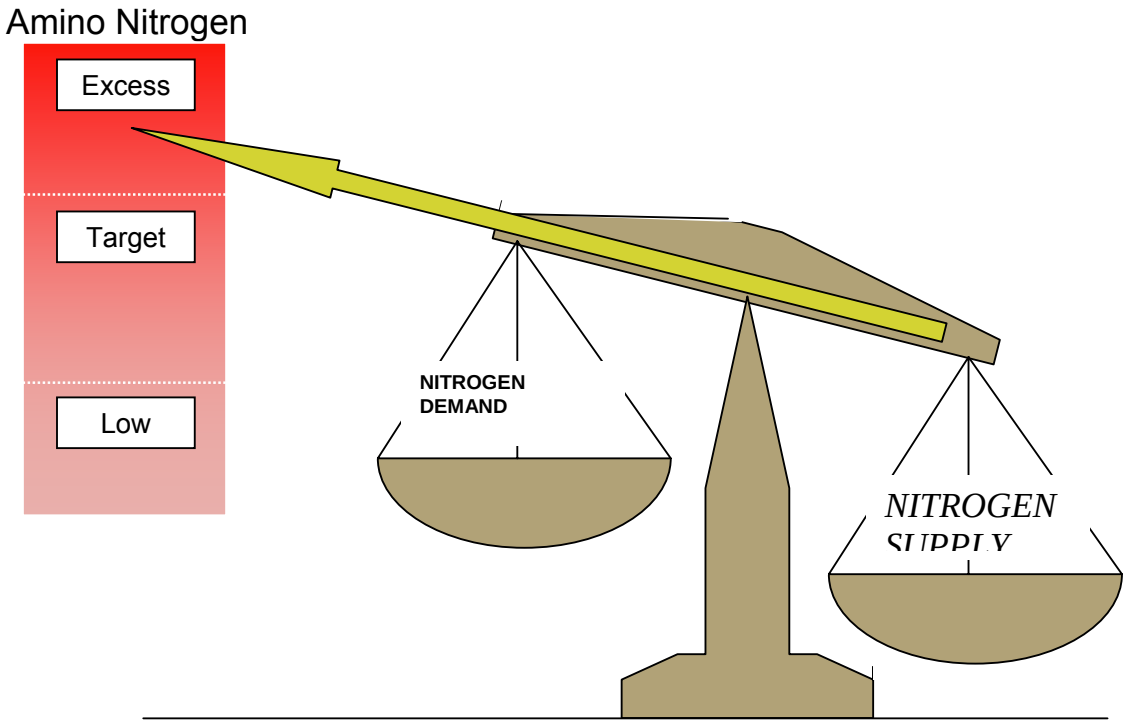


When the nitrogen demand of a crop and the nitrogen supplied to the crop are in balance, the amino nitrogen will be in the target range.

A small crop with a low nitrogen demand supplied with a low amount of nitrogen will be in balance. A large crop with a heavier nitrogen demand will also be in balance if it is supplied with more nitrogen.

Factors that influence **NITROGEN DEMAND**	Factors that influence **NITROGEN SUPPLY**
• Crop Size – weather – water logging – crop lodging – location – ratoon age – pest damage – disease incidence – other nutritional constraints • Possibly variety	• N fertiliser inputs (rates, forms, timing and methods) • Soil N sources (soil type and history) – mineral N residues – mineralisation • Mill wastes • Crop residues • Other minor N sources (rainfall, fixation) • N loss processes – leaching, denitrification, run off, volatilization

Excess Amino Nitrogen

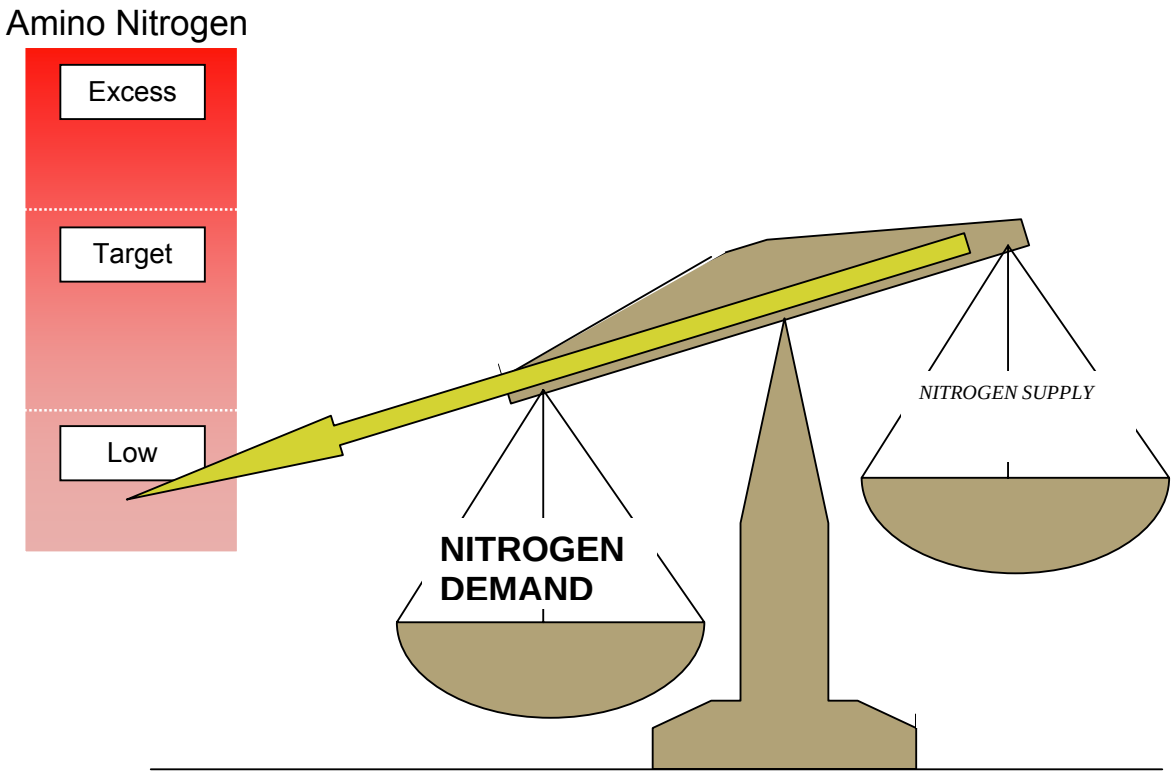


When amino-N is in the excess range it indicates nitrogen supply out-weighed nitrogen demand by the crop. This could result from growing a small crop, or over supply of nitrogen.

Excess amino nitrogen means nitrogen was wasted because there was more available than the crop needed. Excess amino-N suggests nitrogen may leave the farming system (because it is in excess), possibly contaminating surface or ground waters.

Factors that cause a light **NITROGEN DEMAND**	Factors that cause a heavy **NITROGEN SUPPLY**
• Any factor that causes small crop size: – Unfavourable weather – stool damage – water logging – crop lodging – very old ratoon – excessive pest damage – high disease incidence – other nutritional constraints • Loss of N fertiliser after application: – Leaching to ground water – Volatilisation to atmosphere – Run off to surface water – Denitrification to gases – Consumption by soil organic matter • Possible variety influences	• High N application for current crop • High residual N from high N application to previous crop • Mill mud or other wastes in addition to normal N fertiliser rate • Fallow ground prior to planting • Growing legumes prior to planting • Crop residues incorporated prior to planting • Soil N sources (influenced by soil type) – mineralisation – mineral N residues • Some minor N sources (rainfall, fixation)

Low Amino Nitrogen



Low amino-N indicates crop demand for nitrogen outweighed nitrogen supply.

Large crops have a higher demand for nitrogen than smaller crops. Low amino nitrogen will occur when not enough nitrogen is supplied to the crop, or when nitrogen is lost from the system before the crop is able to take nitrogen up.

Factors that cause a heavy **NITROGEN DEMAND**	Factors that cause a light **NITROGEN SUPPLY**
• Any factor that causes large crop size: – Favourable weather – Irrigation – Plant/Replant crops – Good stools – Adequate nutrition – Controlled pests and diseases • Possibly variety	• Low N fertiliser inputs • Applying unavailable forms of fertiliser • Incorrect fertiliser placement • N losses after application: – Leaching to ground water – Volatilisation to atmosphere – Run off to surface water – Denitrification to gases – Consumption by soil organic matter

Implement the process

Implementation involves bringing all of the design processes together and conducting the extension program.

Evaluate the process

Evaluation of the success or otherwise of this extension component to an entire mill area should be an ongoing process from the beginning of the implementation. Each of the objectives identified earlier should be evaluated against, however it is probably too difficult and expensive to evaluate the two highest level objectives ("desired change in grower practice is the more efficient use of nitrogen fertilisers to raise sugar industry profitability and minimise negative environmental impact from losses" and "end result objective is the maintenance of soil fertility and sustainable sugar productivity in the long term through improved fertiliser management"). The lower level objectives should be evaluated and if they are met, supposition should suggest processes are in place to work towards achieving these two high level objectives.

Ongoing evaluation against the lower level objectives is important because it lets the researchers know how closely they are following the planned process. If through evaluation the process being undertaken deviates significantly from the plan, steps should be taken to ensure the extension program is still achievable. This is more desirable than discovering through evaluation at the completion of the program that it was not successful because of something that could have been rectified earlier.

Appendix 16: Report on how sampling from cane supply in 2001 harvest (Year 3) has been used to test and improve NIR calibrations.

1. Investigating amino-N determination

How amino-N is derived needed to be investigated to explain if this was influencing the inability of NIR to develop a calibration equation for amino-N. Project CTA029 has shown NIR can be calibrated for amino-N in juice. The NIR being used for this project is scanning the fibrated cane supply as it is entering the number 1 mill. The amino-N values used to develop calibrations for NIR must accurately reflect the amino-N of the whole cane supply NIR is scanning.

1.1 Previous NIR calibrations for amino-N

Juice sampled from the cane supply in 2000 was used to test the NIR calibration equation for amino-N and has been reported in milestone report 5. The validation plots reproduced below for Mulgrave and Mossman (Appendix 12, Figures 3 and 4) showed a very poor calibration for NIR.

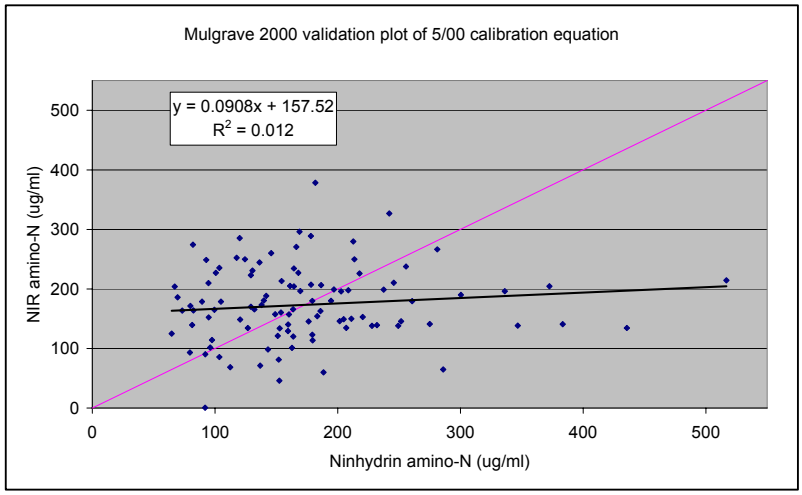


Figure 1: Validation plot of May 2000 global calibration equation, Mulgrave 2000.

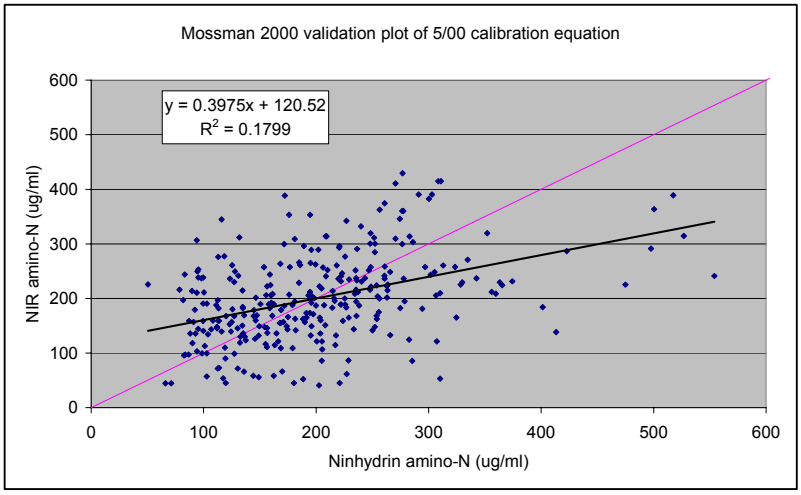


Figure 2: Validation plot of May 2000 global calibration equation, Mossman 2000.

Before collecting juice from the 2001 cane supply it was deemed necessary to investigate the reasoning behind the poor performance of the calibration equation given the equation was developed from a pool of 1 064 amino-N samples, sufficient to remove any inherent NIR scatter.

NIR scans the shredded cane in the number 1 mill feed chute. Because amino-N is determined from juice, samples of first expressed juice are collected and analysed for amino-N. These first expressed juice samples are used to calibrate NIR. It was felt that because NIR is scanning a different product to that used for determining the calibration samples there may be some error introduced at this point.

This investigation studied the amount of hydraulic pressure used to extract the juice sample, the degree of preparation of the samples prior to applying hydraulic pressure, a revised HPLC analysis of juice samples for direct comparison with ninhydrin data and the effect of the filtering process used in ninhydrin assays on amino-N.

1.2 Juice collection

All juice collected for NIR calibration equation development and validation has been first expressed juice. First expressed juice was deemed appropriate because each rake is sampled (and a corresponding rake scan from NIR taken) and collection is relatively straight forward.

In previous amino-N work field samples of cane are cut and juice extracted through jeffco cutter-grinding the cane and extracting juice from this sample with a carver press. This method of extracting juice is necessary because trial work rarely produces enough cane required to constitute an individual rake at the mill, meaning first expressed juice is not able to be collected.

It was hypothesised amino-N extracted from first expressed juice might not accurately represent the amino-N of the cane supply. This could explain why NIR is unable to develop a calibration equation from amino-N determined from first expressed juice.

Possible differences between first expressed juice and jeffco/carver extraction were investigated. There are two possible sources that may cause differences in amino-N extracted, namely the amount of hydraulic pressure applied to extract juice and the level of cane preparation prior to applying the hydraulic pressure.

1.2.1 Level of hydraulic pressure

The carver press exerts a very high pressure when extracting juice (10 000 kg for 60 sec) when compared with the pressure used to extract first expressed juice. Juice extraction from the carver press of jeffco cutter-ground cane closely reflects the quantity of juice that can be expected from the entire milling train, significantly greater than first expressed juice. If certain amino acids are preferentially bound to the cane supply, and need a high extraction pressure to be removed in the juice, this could be a possible source of variation in measured amino-N.

To investigate this a trial varying the hydraulic pressure applied to cane supply with the carver press was carried out. Mature sticks of cane were sampled from the field prior to harvest and fibrated with the jeffco cutter-grinder. Three hydraulic pressures were applied to the fibrated samples and amino-N of the juice determined. The lowest hydraulic pressure was equivalent to a squeeze less than is applied to extract first expressed juice and the highest was the standard carver press. Figure 3 shows results from four varieties.

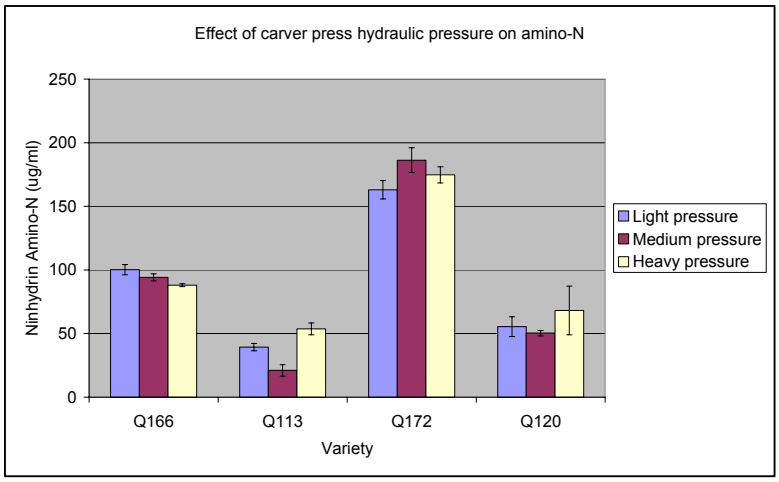


Figure 3: How the hydraulic pressure influenced the extractable amino-N in four samples where the samples were jeffco prepared and carver pressed at three hydraulic pressures.

The amount of hydraulic pressure applied to cutter-ground cane to extract juice did not appear to influence the amino-N of the juice. This suggests amino acids removed from cells during the fibration process are not binding to the cane supply and are freely extractable in the juice.

1.2.2 Level of preparation

Cane in the number 1 mill feed chute has passed through the mill shredder prior to juice extraction. This level of preparation is less than that provided by the jeffco cutter-grinder, that is a lower number of cells have been ruptured. If amino-N bound in the unruptured cells is proportionally higher than the amino-N of juice from opened cells this would have an impact on the ability of first expressed juice to accurately provide a measure of amino-N for the whole cane supply.

Experimentation was conducted where shredded cane was collected from across a rake of cane from the number 1 mill feed chute. Half of this sample was then prepared further by passing through the jeffco cutter-grinder and juice from both samples extracted with the carver press (both high and low hydraulic pressures). First expressed juice from the rake was also collected and all samples analysed for amino-N.

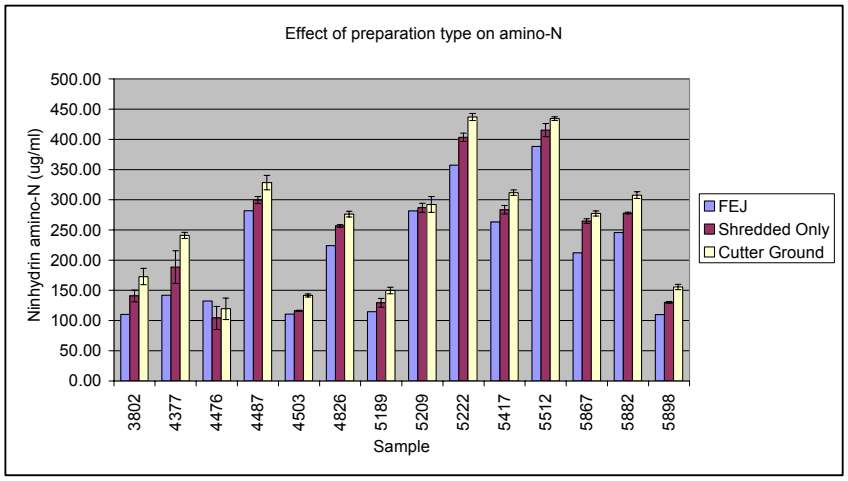


Figure 4: Effect of cane preparation on extraction of juice amino-N. Shredded and cutter-ground samples were heavy carver pressed.

Figure 4 shows the amino-N of the two cane preparation levels and first expressed juice when the high hydraulic pressure was applied. As the level of preparation increased from shredded cane to cutter-ground cane the extraction of amino-N generally increased. This supports work presented in project CTA029 which showed small mill extraction significantly underestimates amino-N concentrations of juice derived from jeffco/carver. There is evidence from stem partitioning that sugarcane stores higher concentrations of stem amino acids in outer stem tissues and the rind. This could explain why increased fibrating of stem tissue increases the amount of amino acid extracted.

Somewhat surprisingly the amino-N of juice extracted from the shredded cane in the carver press was often greater than the amino-N of first expressed juice. This was repeated when the low hydraulic pressure was used. It is not know what is producing this anomaly but supports the belief first expressed juice can not be used to accurately estimate the amino-N of the cane supply.

1.3 Amino-N calculation

Amino-N is a measure of the amount of nitrogen attached to amino acids in the cane juice. Amino-N can be determined through HPLC analysis by counting the number of 19 individual amino acids in juice and then adding up the number of nitrogens associated with those amino acids (individual amino acids contain between 1 and 4 nitrogens). A secondary method for determining the number of amino acids is the ninhydrin reactive test. The ninhydrin method counts the total number of amino acids in a sample of juice, however does not differentiate between individual amino acids. A conversion equation is used to convert results of ninhydrin analysis to amino-N based on relationships between the two determined through HPLC analysis.

1.3.1 Evaluation of the ninhydrin reactive test

During the 2001 season 82 samples of jeffco/carver extracted juice and the corresponding first expressed juice from Mulgrave mill were analysed via HPLC for amino acids. These samples were chosen because NIR had taken very good scans of each rake as it passed through the mill.

Figure 5 shows the ninhydrin assay detected quantities of amino acids closely corresponds to the HPLC determined quantities. This indicates the ninhydrin reactive test is a valid method for amino acid determination.

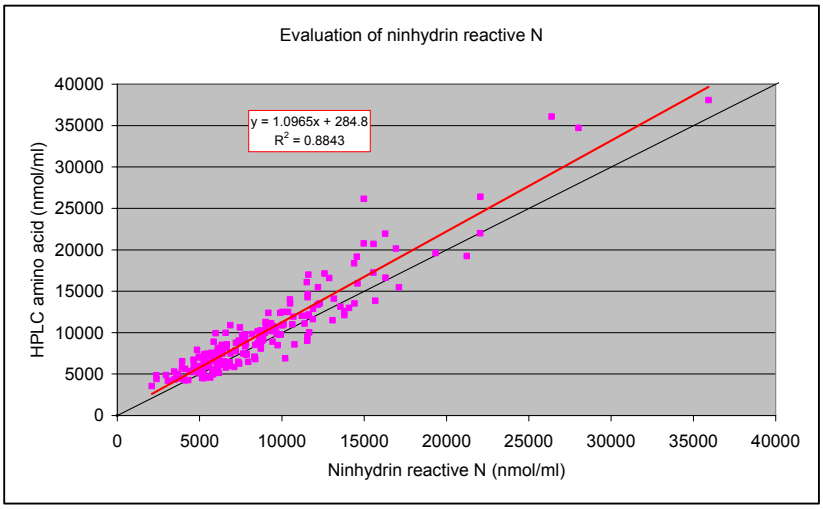


Figure 5: Scatter plot of ninhydrin reactive N count of amino acids vs HPLC count of amino acids.

1.3.2 Evaluation of amino-N conversion equation

HPLC determined amino-N can be plotted against ninhydrin reactive N to estimate a conversion equation from ninhydrin to amino-N. The previous set of HPLC data taken in 1996 estimated a conversion equation and has been used since.

Figure 6 shows the comparison between amino-N derived from the existing conversion equation and the amino-N derived from the 2001 set of HPLC data. The conversion equation is accurate at low values however as amino-N increases greater than 200 ug/ml some scatter is introduced and the equation is underestimating the quantity of amino-N present.

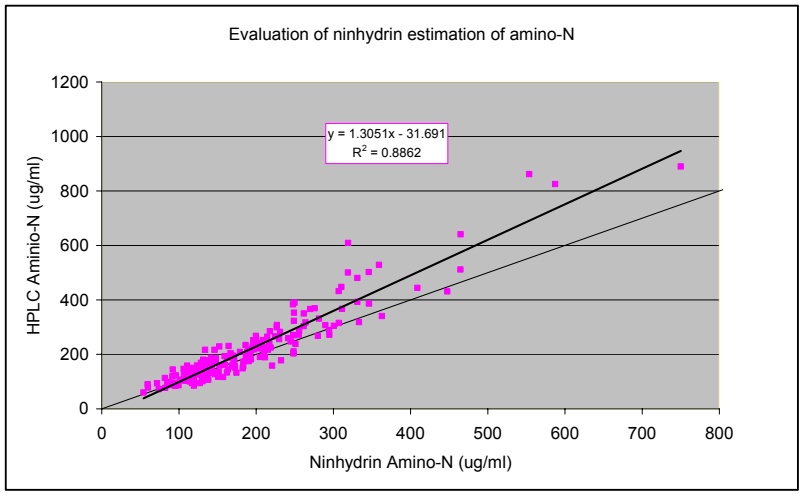


Figure 6: Scatter plot of ninhydrin estimated amino-N (1996 conversion equation) vs HPLC determined amino-N for 2001 data.

Figure 7 shows the 2001 set of HPLC amino-N plotted against ninhydrin reactive N and estimates a new conversion equation. This relationship has a high r^2 value however the conversion equation is significantly different from the one developed in 1996. It is intended to further investigate this conversion equation to ensure future conversion of ninhydrin data to amino-N is accurate.

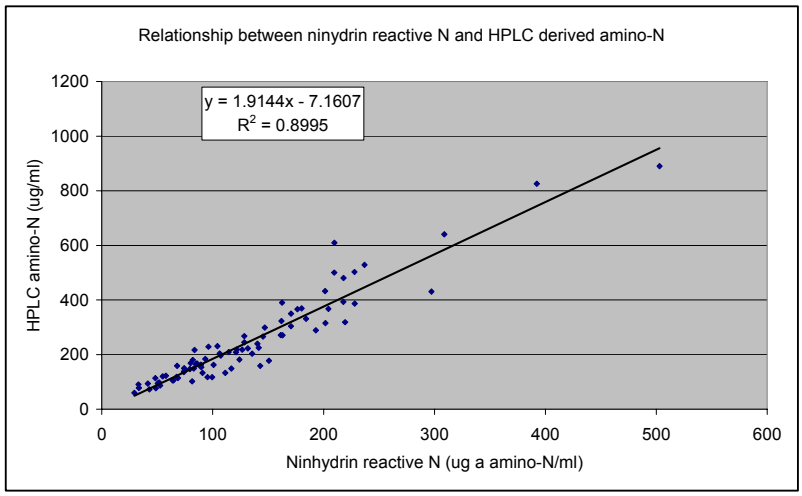


Figure 7: Relationship and estimation of a conversion equation from ninhydrin reactive N to HPLC derived amino-N.

1.3.3 Effect of filtering juice sample

There was some suspicion the use of 0.8 um filters in the juice preparation process prior to ninhydrin analysis may bind some nitrogen containing compounds. The amino-N of juice samples was determined for samples filtered with or without the 0.8 um filter. Figure 8 shows filtering has no effect on amino-N. This finding is significant as early work on NIR of sugarcane juice showed a good calibration equation is possible on juice filtered through 0.8 um filters. This has possible flow on influences for in-line filtering of juice for NIR scanning in the juice lab.

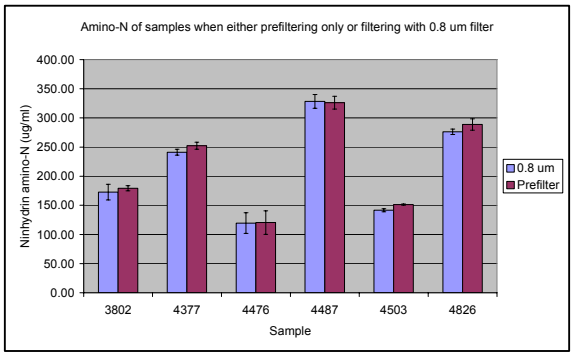


Figure 8: Evaluation of filtering with a 0.8 um filter on amino-N.

2. Improving the NIR calibration

Currently a calibration equation for determining amino-N by NIR is unable to be developed. The information presented above suggests it is possible to use samples that accurately represent the amino-N of the cane supply NIR is scanning. These samples have been used to develop a calibration equation for amino-N.

2.1 Developing a new NIR calibration equation

2.1.1 Ninhydrin determined amino-N

An attempt was made to develop a new calibration equation based on jeffco/carver samples that had amino-N determined by the ninhydrin reactive method and using the existing conversion equation to amino-N. Figure 9 is the validation curve for that calibration plot.

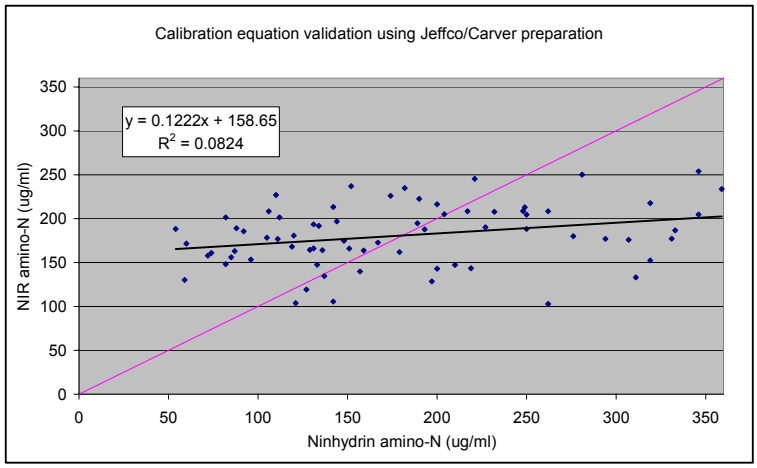


Figure 9: Validation plot for 2001 calibration equation developed from jeffco/carver samples (1996 conversion equation), Mulgrave 2001.

No calibration was possible even when the juice used in calibration development accurately reflected the amino-N of the cane supply. There was obviously a problem in the conversion to amino-N. A similar validation plot was produced when first expressed juice was used.

Figure 10 explains how this was to be expected. The relationship between first expressed juice derived amino-N and jeffco/carver samples is linear and very close to a 1:1 relationship. The jeffco/carver determined amino-N values are simply higher than the first expressed determined samples, and the values are simply shifted up. Shifted values such as these will not produce a better NIR calibration.

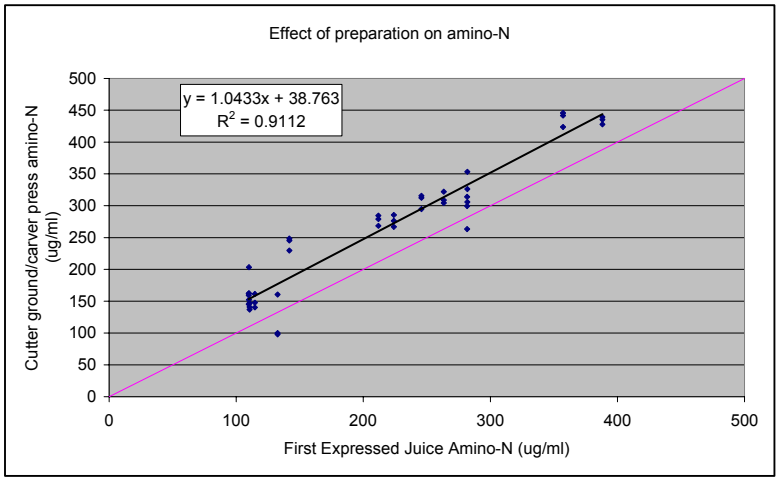


Figure 10: Relationship between first expressed juice derived amino-N and jeffco/carver derived amino-N.

Using amino-N values determined from the 1996 conversion equation from ninhydrin reactive N to amino-N can not produce a valid calibration even when the juice accurately represents the amino-N of cane supply (jeffco/carver). This effectively means the problem lies with the determination of amino-N from the conversion equation.

2.1.2 HPLC determined amino-N

As HPLC analysis was conducted on 82 samples to evaluate the ninhydrin method these samples were used to develop a NIR calibration equation for amino-N. Figure 11 shows the validation curve for these samples.

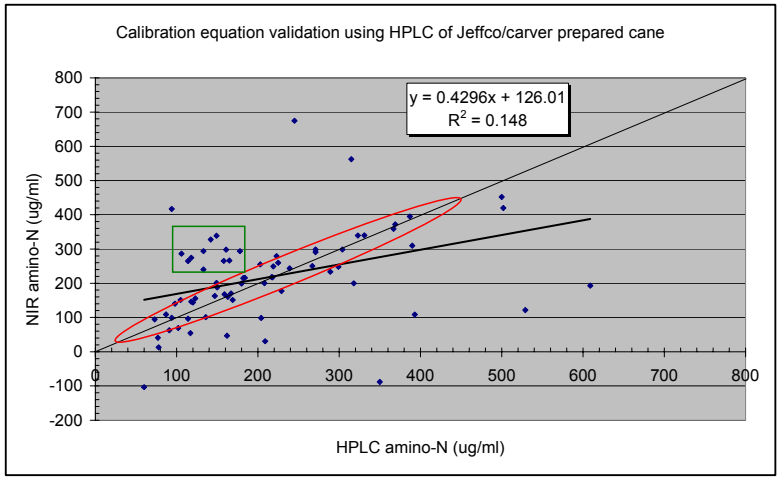


Figure 11: Validation plot of 2001 HPLC calibration equation developed from jeffco/carver HPLC samples, Mulgrave 2001.

Only 47 samples are used in this calibration equation. When a small number of samples such as this is used there will be some inherent scatter in the NIR validation plots corresponding to samples whose NIR scan profile has not been used in the calibration. This is obvious in this validation as NIR has estimated two amino-N values as negative! This scatter can disappear when more samples are added to the calibration equation covering a greater proportion of the population of samples NIR will scan.

The important observation to make with this validation plot is there is a core of samples that lie on the 1:1 line between NIR and laboratory determined amino-N. The presence of this core is usually a good sign that further sample collection will produce a valid calibration equation.

Another note to make is the cluster of samples highlighted by the green box where NIR overestimates the amino-N of a particular group of samples with a similar laboratory determined amino-N. Experienced NIR technicians suggest clusters such as this are common in calibrations from a small sample number. Some common link between this type of sample cluster usually exists and when explained, or often with the addition of more samples to the calibration equation, the cluster of samples move to the 1:1 line.

Figure 12 shows the deviation of NIR derived amino-N from HPLC determined amino-N. It clearly shows the majority of samples are close to the 1:1 line (less than 50 units deviation) and identifies the cluster discussed above (overestimating around 150 units).

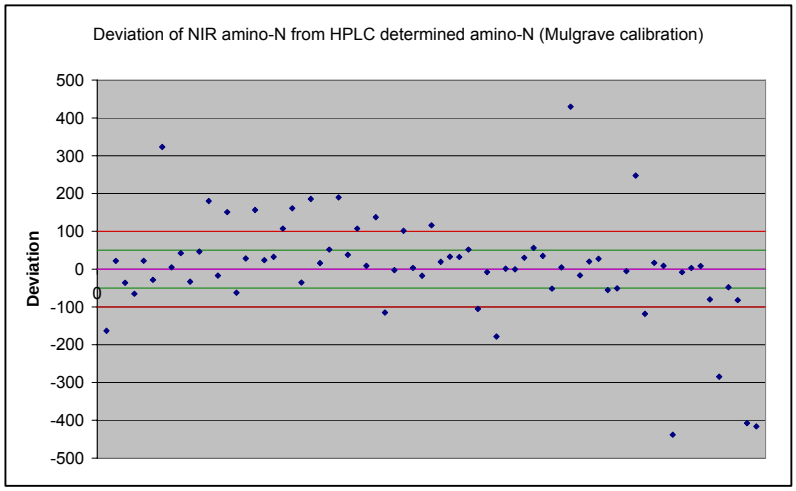


Figure 12: Extent of NIR estimation deviation from HPLC derived amino-N for Mulgarve 2001 samples.

2.1.3 Usefulness of ninhydrin

The ninhydrin reactive test was shown to be valid in measuring the number of amino acids in a juice sample (Figure 5). Using the new conversion equation for amino-N the ninhydrin derived amino-N values were used to validate the above developed calibration equation. Figure 13 shows the validation plot.

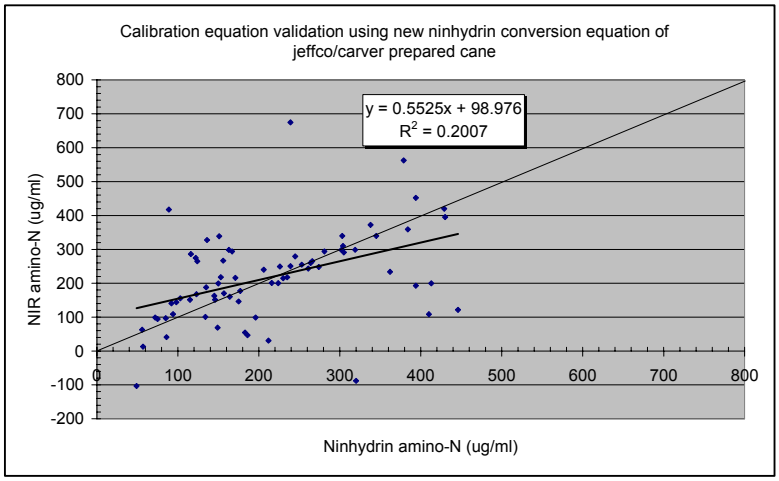


Figure 13: Validation plot for ninhydrin determined amino-N on HPLC calibration equation, Mulgrave 2001.

Again this validation plot shows a core of samples along the 1:1 line and the cluster discussed above. This plot suggests there is a role for ninhydrin determined amino-N values in calibration development and validation.

3. Conclusion

NIR has been unable to develop a calibration equation for amino-N when first expressed juice is used in estimating the amino-N of the cane supply. An investigation into determination of amino-N discovered the level of cane preparation influenced the extraction of amino acids and consequently the measurable amino-N. This investigation also led to an evaluation of the conversion equation from ninhydrin reactive N to amino-N. An alternate conversion equation based on a dataset collected in 2001 was developed and evaluated.

HPLC derived amino-N of 47 samples was used for a preliminary calibration of NIR. This calibration shows good promise and is much better than the 'shoot gun' scatter NIR calibration validations previously showed.

This investigation into NIR determination of amino-N suggests a calibration is possible when the amino-N of the cane supply NIR is scanning is accurately determined. Juice extracted from carver pressing jeffco cutter-ground prepared cane and analysed by HPLC is an accurate way of determining the amino-N of the cane supply. There is promise ninhydrin determined amino-N also has a valid role in NIR calibration and validation.

Additional samples of jeffco/carver extracted juice analysed by HPLC will be collected to further investigate the conversion equation from ninhydrin reactive N to amino-N and will also be added to the NIR calibration dataset. A significant number of samples will be collected and analysed for these purposes.

Appendix 17: Report on how sampling from cane supply in 2002 harvest (Year 4) has been used to test and improve NIR calibrations.

Sample collection and analysis

During the 2002 crushing season a total of 355 samples were taken for amino-N determination (150 from Mossman, 142 from Mulgrave and 63 from Tully). Each of the 355 samples collected include a juice sample of first expressed juice (FEJ), a Jeffco/Carver juice sample (JC) and a sample of cane fibre processed through the Jeffco cutter grinder.

The first expressed juice and Jeffco/Carver juice samples have had amino acid concentration determined via the ninhydrin reactive test. Amino-N has been calculated using both the old conversion equation (1996) and the recently developed conversion equation (2001). An attempt to calibrate NIR from both these amino-N values has been made.

The Jeffco/Carver juice samples have had individual amino acids concentrations determined via High Performance Liquid Chromatography (HPLC). Amino-N can be accurately calculated from these samples because the number of nitrogens associated with each amino acid is known. The relationship between the HPLC determined amino-N and the ninhydrin reactive N produces the conversion equation for converting ninhydrin reactive N to amino-N. This procedure during 2001 (outlined in appendix 16, milestone report 7.1) resulted in the development of the 2001 conversion equation. HPLC samples from 2002 are being used to validate, and where necessary improve, the 2001 conversion equation.

The collected fibre samples are being treated with methanol to extract all soluble substances from the cells, including amino acids. Methanol extracted juice from the fibre will be analysed via the ninhydrin reactive method. Results of ninhydrin reactive N will be compared between these methanol extracted samples and the Jeffco/Carver juice samples. This comparison is being made to determine if any variation in amino-N measurements is potentially being introduced because the Jeffco/Carver technique is failing to extract all, or a constant proportion of, amino acids.

Attempts have been made since the 1999 crushing season to calibrate NIR for amino-N, and this 2002 season is the last attempt at making a calibration under this SRDC project. For this reason a back up measurement has been deemed necessary by the project team to provide an outcome to growers should amino-N prove too difficult to calibrate for using the Cane Analysis System NIR. During early NIR developmental work total N was calibrated for using the Cane Analysis System used in the mills today. The fibre samples collected from each sample will have total N determined, and an NIR calibration attempted. The projects NIR technical team believe a valid and robust calibration can be made. While this is not the desired output from the project, the option of having ‘some’ measure of nitrogen is better than none. Relationships between total N and amino-N are well established and how growers can use total N information to better manage nitrogen on farm is known.

NIR calibration from 2002 collected samples

Usually a ‘global’ equation is developed during the NIR calibration process where one calibration covers all NIR units in all mills. An alternative to this is developing separate calibrations for each mill. Due to the difficulties in developing a calibration for amino-N separate calibrations have been attempted for each mill for 2002. The calibration that looks the most promising can then be analysed further to identify any areas where the equation can be improved further. The samples for HPLC analysis have come from this dataset. Once improvements are identified and made, these procedures are applied to the other samples in an attempt to develop a robust global equation.

Data from Mossman during 2002 developed the best calibration. Figure 1 presents the validation chart for this calibration.

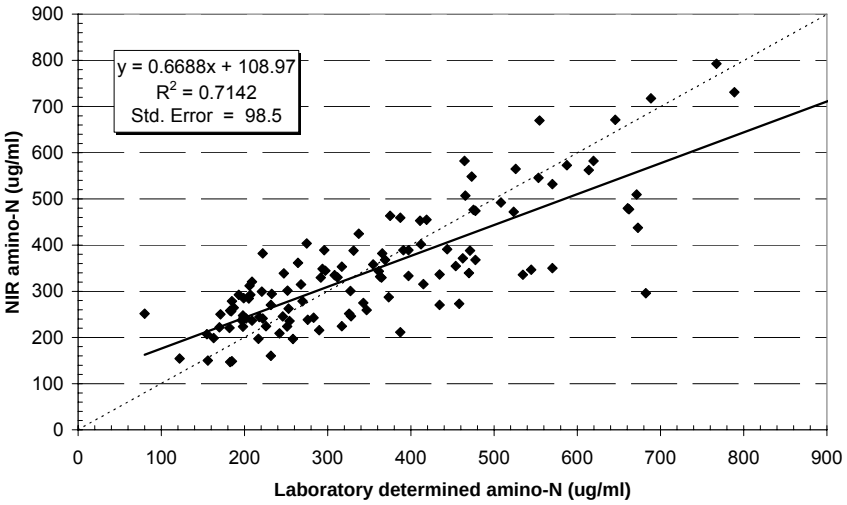


Figure 1: Validation plot of local NIR calibration of amino-N using 2001 conversion equation for Mossman samples, 2002.

Improvements to amino-N calibration equation

Since 1999 the project has been attempting to calibrate the Cane Analysis System NIR for amino-N. The following sequence of charts shows how the validation plots from Mossman mill derived data has improved from 1999 to the present.

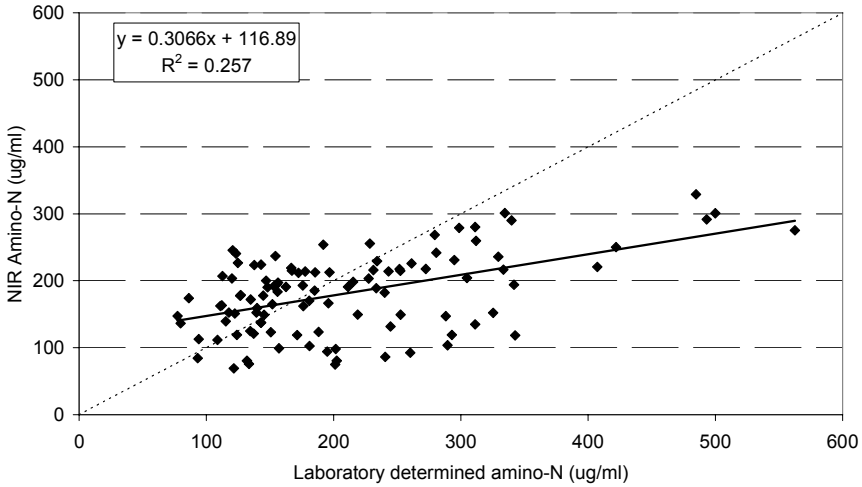


Figure 2: Validation plot of NIR derived amino-N from Mossman mill, 1999.

The 1999 calibration equation validation was taken from 109 samples of first expressed juice. The major limitation of the equation is NIR is not measuring the very high amino-N samples, producing a flat calibration curve.

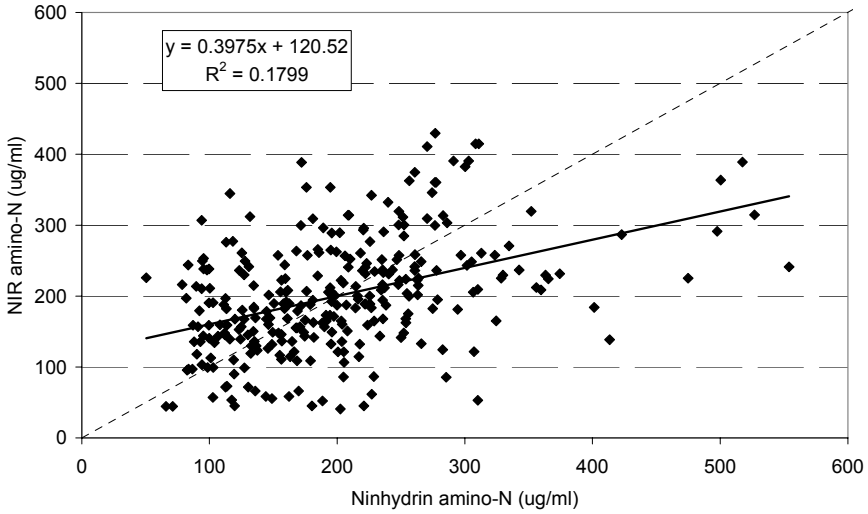


Figure 3: Validation plot of NIR derived amino-N from Mossman mill, 2000.

During the 2000 season a large dataset was developed, with 286 samples collected and used in validation of the recently recalibrated global equation. Once a large number of samples were used in the calibration process it was obvious a calibration using the current technique could not be achieved. This dataset prompted investigative actions into the amino-N determination technique and how it could be limiting the ability of NIR to develop a robust calibration equation.

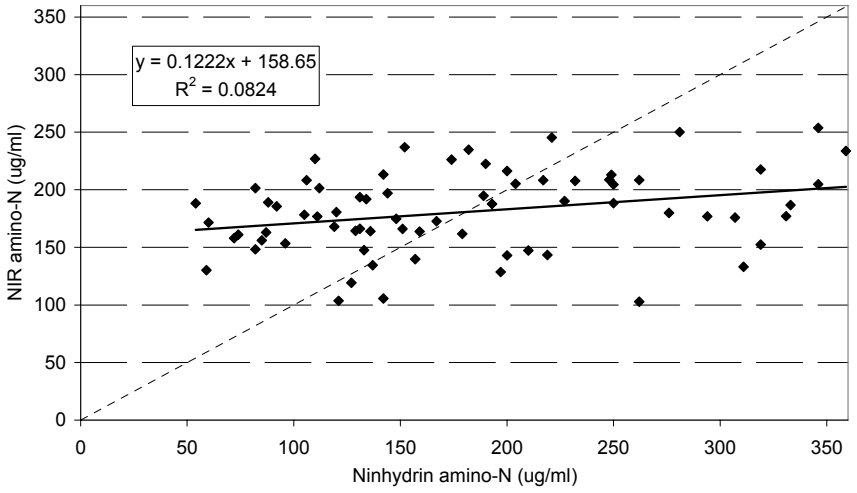


Figure 4: Validation plot of NIR derived amino-N from Mossman mill, 2001. Calibration made using existing amino-N determination technique on first expressed juice.

During the 2001 crush 70 samples of first expressed juice were collected from Mossman and used in a validation check of the existing calibration. Figure 4 highlights the deficiencies of this calibration. After extensive investigations a new calibration was developed using

Jeffco/Carver extracted juice and a new amino-N conversion equation from ninhydrin reactive N.

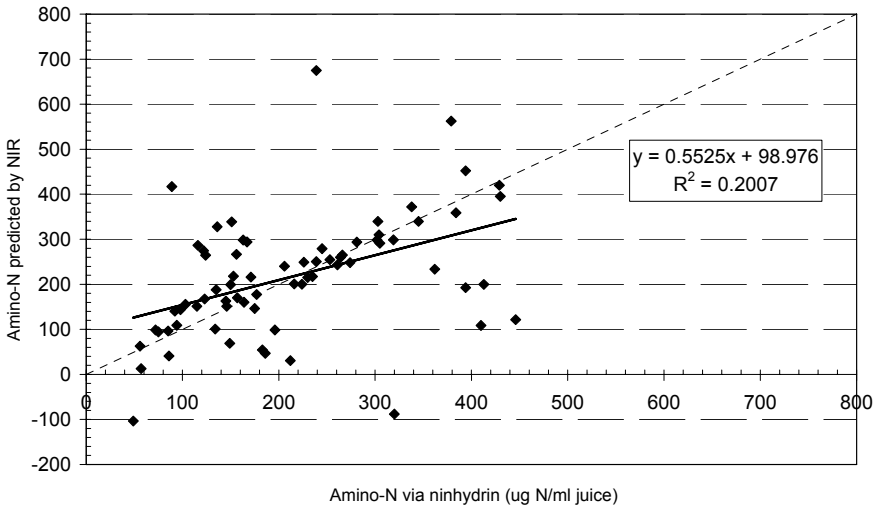


Figure 5: Validation plot of NIR derived amino-N from Mossman mill, 2001 using Jeffco/Carver juice and new (2001) ninhydrin reactive N conversion equation.

The same 70 samples from Mossman collected in 2001 are presented in figure 5 validating the new calibration equation. This is an improvement on previous attempts at calibration, however there is still evidence NIR is having difficulty measuring all high amino-N values.

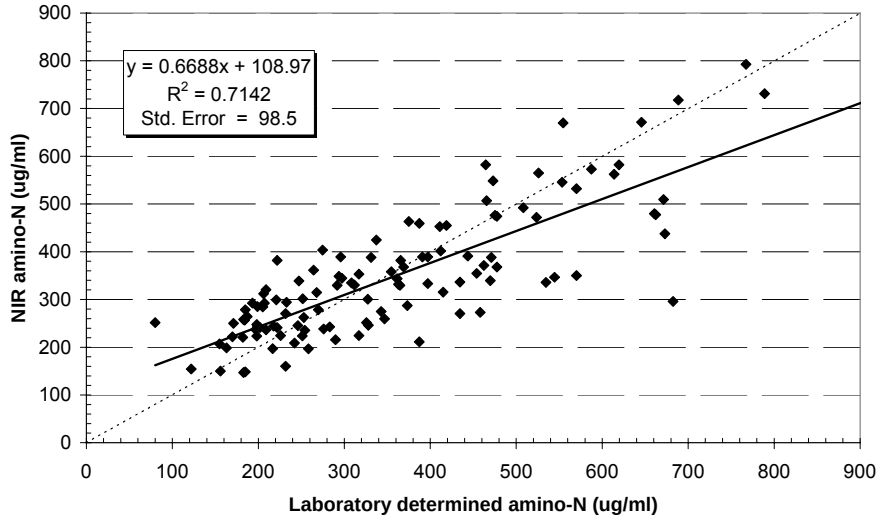


Figure 6: Validation plot of NIR derived amino-N from Mossman mill, 2002.

During the 2002 crush 150 samples were used to calibrate a new equation with figure 6 presenting the validation of that equation. This is an improvement on any of the other calibration equations developed so far in the project, with potential for further improvement once HPLC and methanol extraction samples have been analysed and used to improve the technique.

Appendix 26: Test of reflectance NIR for monitoring amino-N of unfiltered first expressed juice by FOSS PACIFIC.

FOSS PACIFIC

FOSS

Tony Webster *Thursday 3rd May 2001*

Research Agronomist

CSIRO Sustainable Ecosystems

Dear Tony,

This letter reports on the feasibility of analysing amino-nitrogen in cane juice by Near-Infrared (NIR) Spectroscopy.

The samples were measured in transmission on a Foss-NIRSystems model 6500 with a beverage module set at a pathlength of 1mm. 32 scans of the sample were referenced to 32 co-added scans of the air reference to produce the resultant absorbance spectra. The spectral region scanned was 400-2500nm using a Lead Sulphide detector for the NIR region (1100-2500nm) and a silicon detector for the visible region (400-1100nm). The sample was placed in a plastic beaker to allow for immersion of the probes in the liquid. The sample was mixed before analysis to make sure that a representative sample was analysed by the NIR for each sample.

The absorbance (Log1/T) spectra for the juice samples are shown in **Figure 1**. As can be seen there is significant baseline variation between samples which if left uncorrected would cause problems during the NIR calibration procedure.

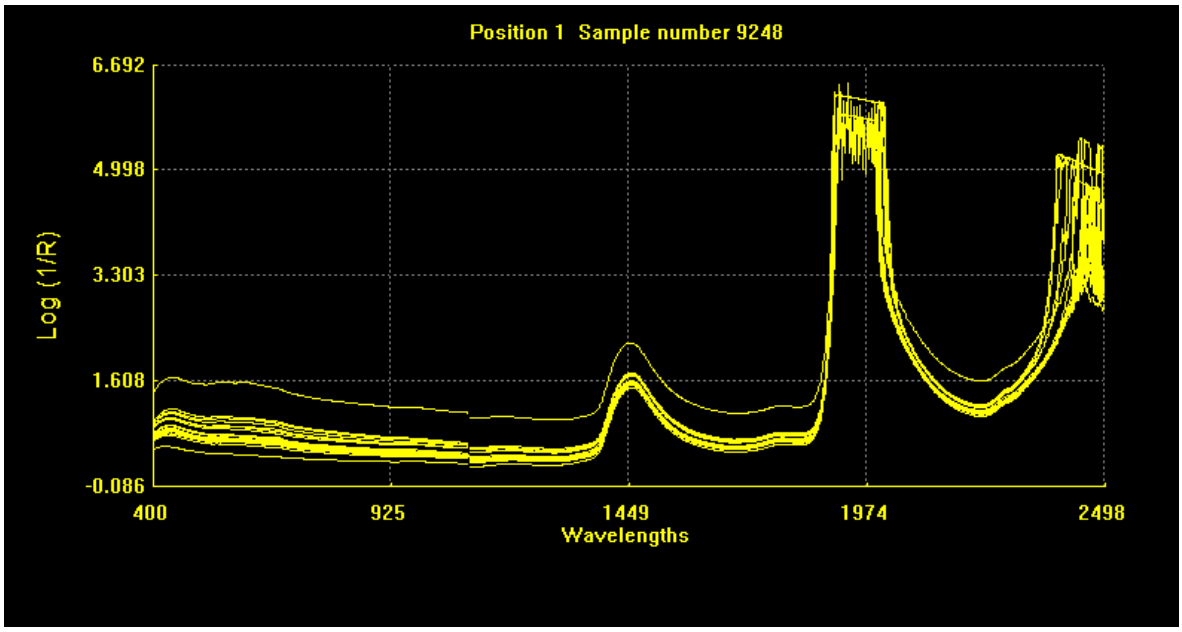


Figure 1: Raw spectra of sugar juice samples

As seen in the spectra, the region above 1800nm was saturated and noisy. This was due to the large water peaks. Noise can also be seen at these wavelengths. For these reason wavelengths above 1800nm were not used in the calibration development. Math treatments and scatter corrections are commonly applied to raw spectral data to minimise particle size and baseline effects and to enhance spectral information. **Figure 2** shows the juice samples with a SNV and Detrend scatter correction and a 1st derivative. SNV stands for standard normal variant and removes the skew from spectra, Detrend removes any polynomic curvature that is present in the spectra. The first derivative changes peak maxima to origin points and origin points to peak maxima or minima.

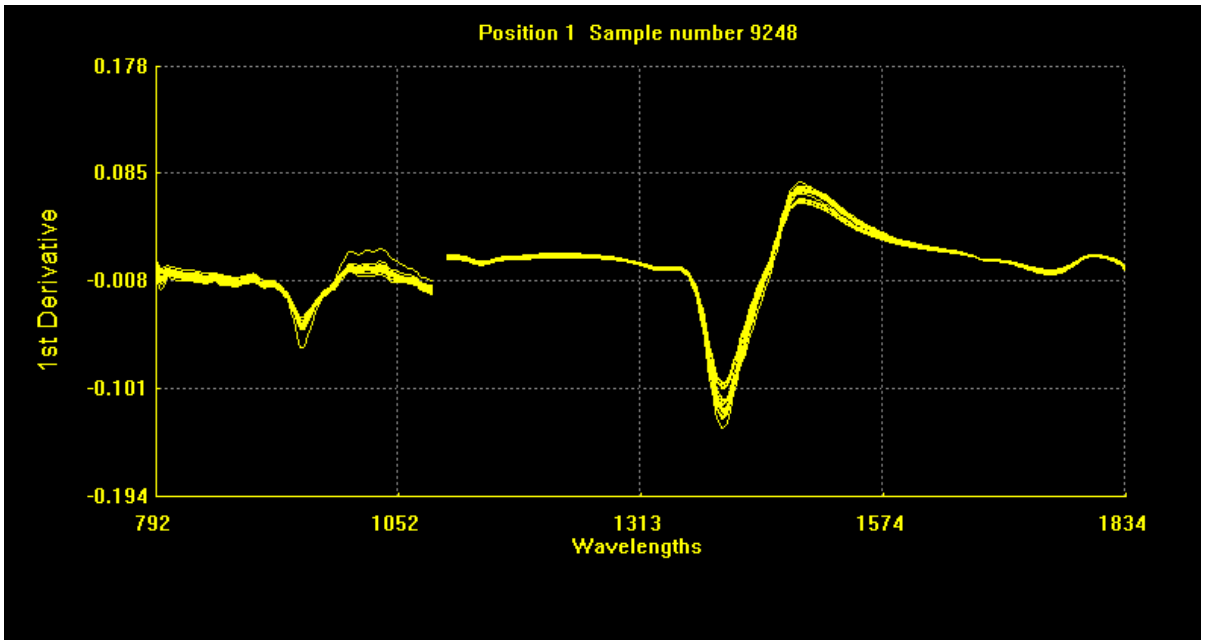


Figure 2: Treated Sugar Juice Spectra

The resulting spectra allow only the variation due to the composition of the sample to be observed.

The calibration for amino-nitrogen was developed using partial least squares regression (PLS). The SECV was calculated to be 170 and the correlation was 0.13. (SECV= Standard Error of Cross Validation is used as this gives a better indication of how the equation will perform). This indicates that the NIR does not agree very closely with the reference values form the lab. This lack of correlation is better observed in **Figure 3** where we plot NIR vs Lab.

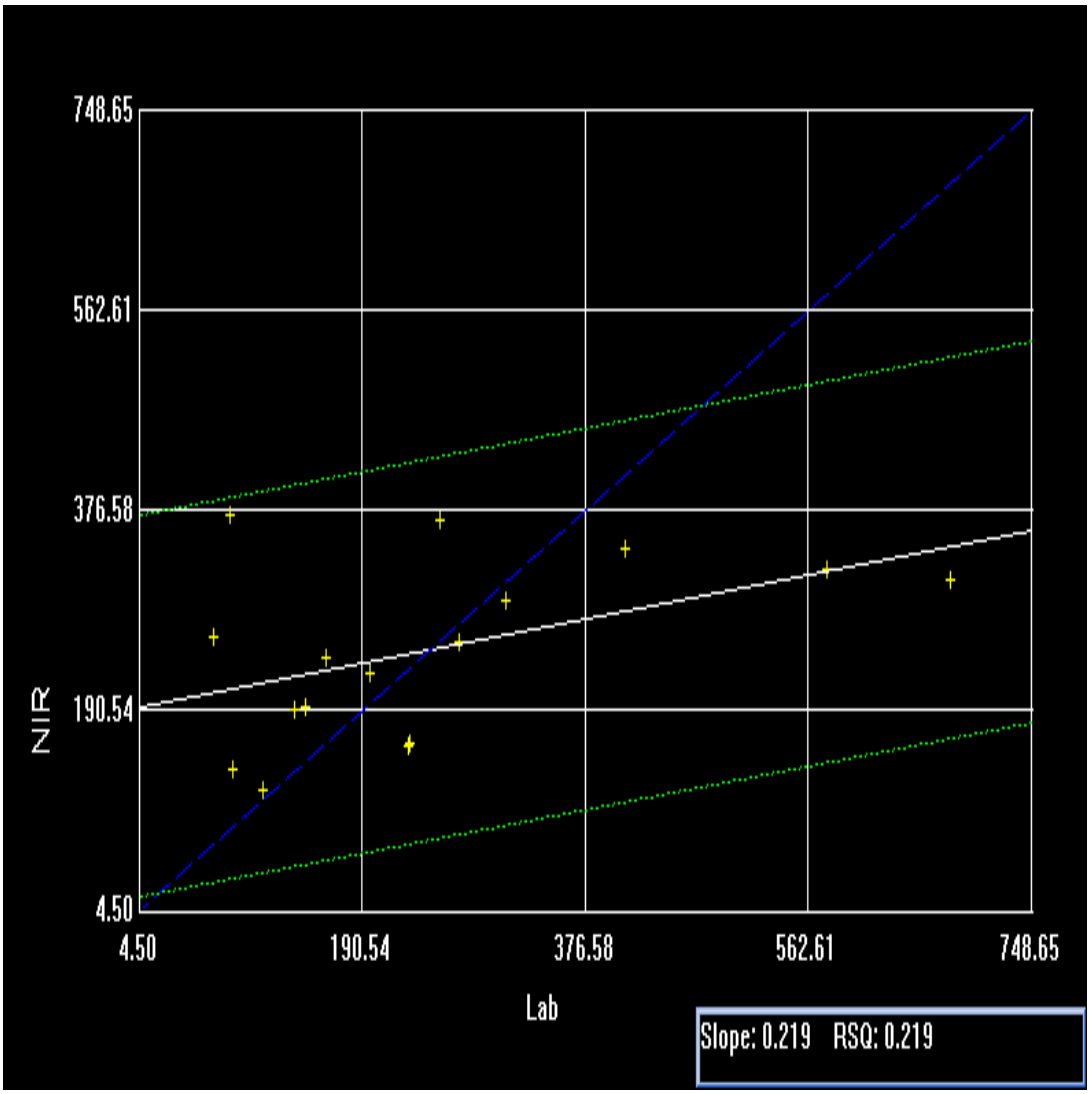


Figure 3: NIR vs Lab for amino-nitrogen in sugar juice samples.

From this graph it can be seen that while the Lab is seeing the samples as containing different levels of amino-N the NIR does not see as much difference between the samples. This is most likely due to the levels being studied. The levels are quite low and NIR is not a trace analysis technique. Other reasons why this measurement does not seem to work could be due to matrix or sample presentation effects. To rule out sample presentation repeatability tests were carried out. This involved running the same sample 5 times and then looking at the standard deviations of the values for each run. The values for this test and the standard deviation are given in **Table 1** below.

Table 1: Repeatability Results

Analysis	Predicted Result
1	191
2	191
3	214
4	227
5	235
SD	**20**

From this table we can see that the SD of 20 is a lot less than the SECV of 170. This means that sample presentation is not a major source of error in the calibration.

Conclusion

From this study it was concluded that NIR couldn't measure the amino-nitrogen levels in dirty cane juice samples. This appears to be due to the low levels being studied and also perhaps due to matrix effects.

If you have any further questions about anything contained within this report please do not hesitate to contact me on either 1300 360 848 or by email jboschenok@foss.com.au.

Best Regards

Jacqui Boschenok

Applications Manager

Foss-Pacific

Appendix 27: Test of transmission NIR to monitor asparagine content of first expressed juice by NIR Technology Australia.

NIR Technology Australia

Analysis of Asparagine Content in Sugarcane Samples Using NIR Spectroscopy.

Scope: The aim of this project was to evaluate the use of a near infrared transmission spectrometer for the determination of asparagine content in sugarcane samples taken directly off the mill.

Samples: 20 unfiltered sugarcane samples were used for the construction of a preliminary calibration. The concentration range of asparagine in the samples ranged from 2μmolml^{-1} to 26μmolml^{-1}, which is equivalent to a range of 400-4000ppm.

Sampling Method: The sugarcane samples were scanned using a Cropscan 2000G NIR analyser in a 30mm pathlength liquid cell, between 720 to 1100nm. Unfiltered samples were homogenised by shaking and the sample was injected to the cell.

The samples were then clarified by centrifuging them at 5000rpm for 15 minutes. The supernatant was decanted from the precipitate and the samples were scanned in the same way as the unfiltered samples.

Calibration Statistics:

1) Unfiltered Samples

Reproducibility was poor for these samples due to the particulate matter resulting in highly variable scattering of the transmitted light, see figure 1. Calibrations constructed from this data were inadequate for use as a predictive model, requiring a high number of principal components to describe them. Therefore the samples were centrifuged and a calibration was developed using these samples.

2) Centrifuged Samples

The following calibration statistics were found for this data, spectral data is presented in figure 2.

Number of samples	Principal Components	SED ($\mu molml^{-1}$)	Correlation	SECV ($\mu molml^{-1}$)
38	8	2.8	0.8758	3.8

The above data indicate that a large number of principal components is required to gain a correlation of 0.8758. The SECV shows that the model is not a good predictor as the results are significantly higher than the SED. However, this model is much better than the one developed for the unfiltered samples and perhaps further clarification may produce a better model by reducing the effects of micro-particulate matter.

Conclusion: Although the concentration of asparagine in the samples was sufficient to be quantitatively determined using NIR spectroscopy, it seems the sample presentation is the limiting factor. The model does indicate that a distinction between high and low asparagine content is possible and perhaps the use of filtered samples may lead to better models, as previous studies have shown this to be the case.

Spectral Data:

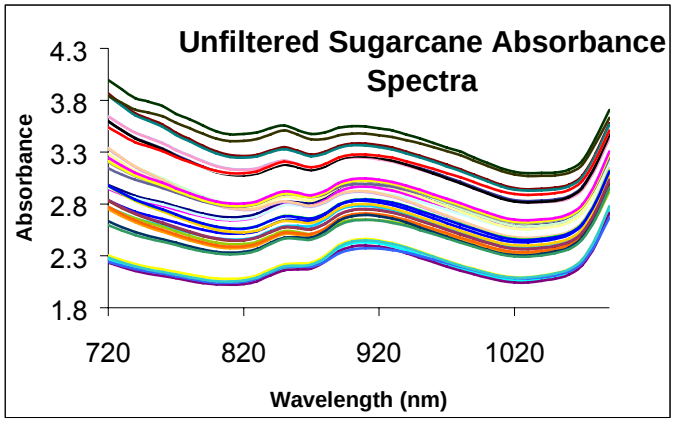


Figure 1: Unfiltered sugarcane spectral data

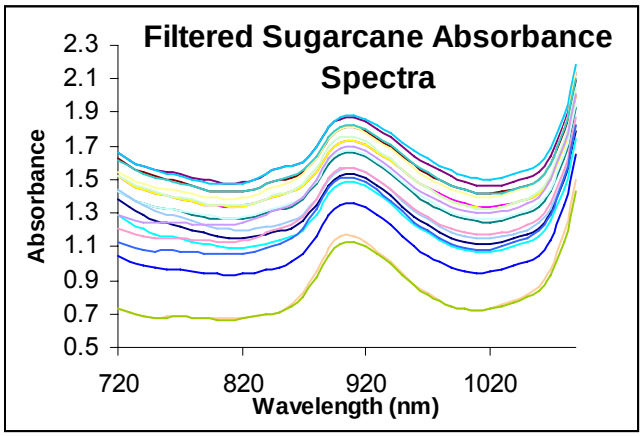


Figure 2: Filtered sugarcane spectral data.

Report by: Brad Swarbrick
Applications Chemist
NIR Technology Australia

Contact: Ph: (02) 9793 8215
Fax: (02) 9790 1552
E-mail: lineart@zipworld.com.au

Date: 27th June 2001.

Sugarcane stem partitioning of amino nitrogen

The sugarcane stem can be viewed as a tube of extremely efficient sink tissue, with leaves at one end and roots at the other (Bieleski 2000). The stem is not only a storage sink for sucrose. Work that has shown the sugarcane stem to be a major sink for excess nitrogen is the basis for monitoring N at the mill projects. The stem cross-section (Fig 1) shows the outer epidermis, a narrow rind region and then vascular bundles embedded in the parenchymatous pith tissue (Dillewjin 1952 and Moore 1987). The rind region of the stem contains approximately 50% of the stems vascular bundles (Walsh et al. 1996) and the accompanying caps of sclerenchyma cells contribute to stem rind hardness (Moore 1987). The pith region consists of mainly thin walled, storage parenchyma cells (Moore 1987).

The rind hardness could be a factor in the efficiency of juice extraction dependent on the degree of stem cell disruption during sample preparation either by the mill shredder or Jeffco cutter/grinder and then by the pressure applied by the N^{o}.1 mill or the hydraulic press. To investigate this, stems were collected from an N rate trial conducted by CSIRO and Bundaberg Sugar at Fairymead, Bundaberg (Dart et al. 2000). The stems were partitioned into sections for the extraction of soluble amino-N components. The concentration of amino-N in the harder rind and nodal regions of the stem may have an impact on extraction efficiency if these areas are more sensitive to storage of excess stem nitrogen then the softer stem pith tissue.

Methods

Juice partitioning experiment carried out as part of the end-of-season hand sampling of Fairymead NUEDI experiment, August 2001. Five stalks were randomly selected from each replicate of three of the N rates treatments (0, 80, and 240 kg N ha^{-1}). Stems were divided into four sections: 1 (top four internodes), 4 (bottom four internodes) and 2 & 3 (remaining middle section of stem divided in two equal sections). Each section was further partitioned into nodal tissue, rind rich tissue and pith tissue. Rind material was sampled by slicing approximately 3mm of outer stem material. Attempted to ensure that only the 'green' tissue layer was removed. This represents a 'rind-rich' sample rather pure rind (epidermis and underlying chlorophyll-containing parenchyma cells and several layers of sclerenchyma cells to the outer vascular bundles).

All juice extracted in 100% methanol with 0.1 mM Norleucine internal standard. Samples were allowed to sit for a minimum of 48 hours in methanol prior to assay. No fresh weight data of the various components was collected. It is therefore not possible to partition actual N balances. Data is presented as ug amino-N per g fresh weight of stem section sampled.

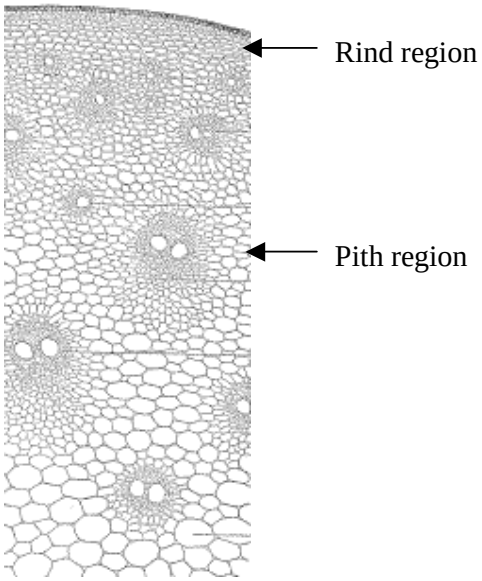


Figure 1 Sugarcane stem section showing the spread of vascular bundles across the stem, the large parenchyma cells of the pith region and the smaller cells of the rind region. Taken from Dillewjin (1952).

Results and Discussion

Generally there is an increase in the concentration of soluble amino-N in all stem tissues with increasing N supply (Fig. 2 a). The exception is the pith tissue between 0 and 80 kg N ha^{-1}. A possible reason could be that the rind and nodal tissues 'fill up' first with excess N prior to overflowing into the pith tissues. It is also interesting that the mature stem sections (base of stem) appear to be more responsive to external N supply. The growing region of the stem (section 1) is the least responsive, suggesting different metabolic functions, i.e., N storage and N metabolism.

The soluble N concentration increases in the harder stem sections (rind and nodes) will have implications for the relative extraction efficiencies of different extraction methods (Jeffco/Carver versus first expressed juice (FEJ)). Such implications may need to be incorporated into the interpretation of results from NIR scanning of shredded cane in the fed chute and calibration of this against FEJ amino-N values.

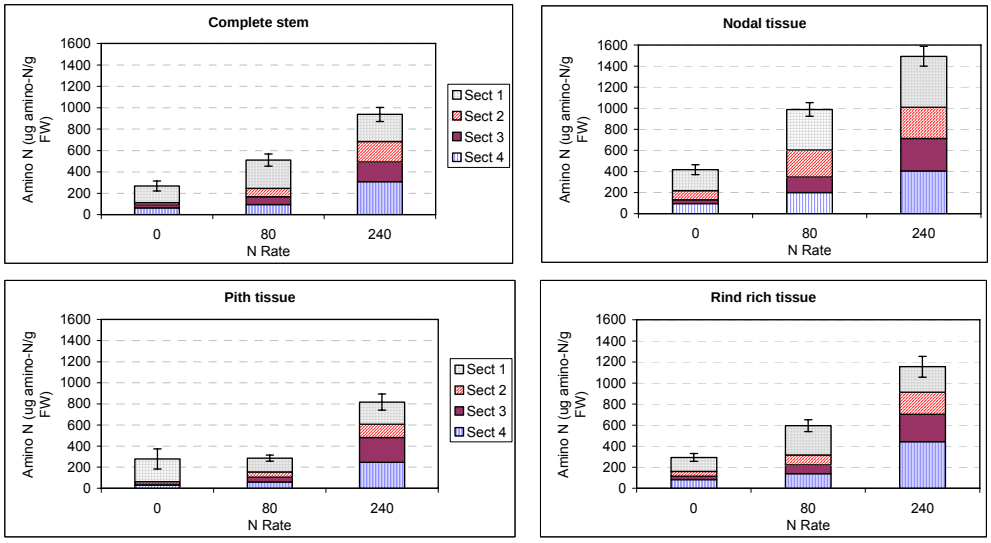


Figure 2 Stem partitioning of sugarcane stem soluble amino nitrogen for the stem column for three N rates (0, 80, and 240 kg N ha^{-1}). Soluble concentrations presented for the complete stem section (A), nodal tissue (B), pith tissue (C), and rind region tissue (D). Error bars represent 1 std. error for the whole stem column.

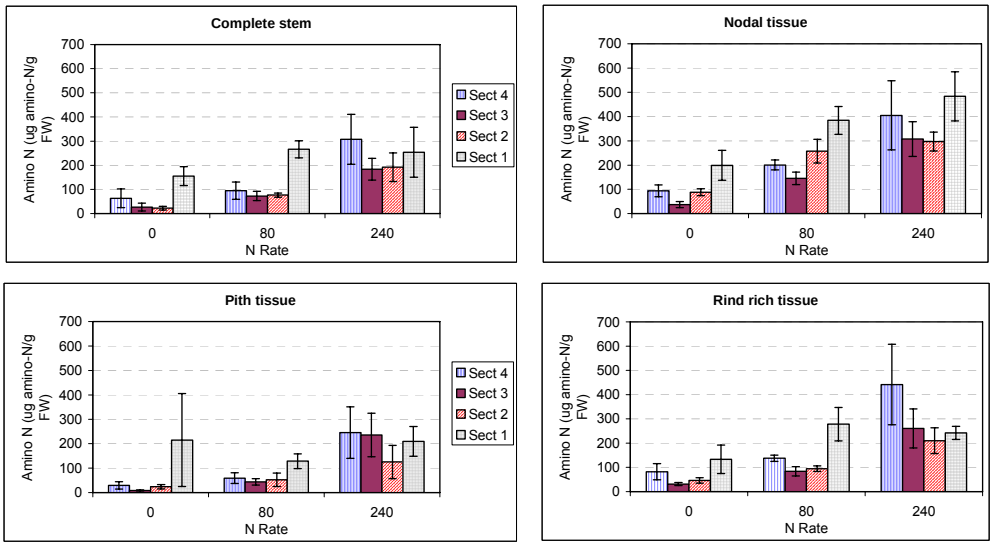


Figure 3 Stem partitioning comparison of the four stem sections each stem was divided into. Error bars represent 1 std. Error.

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Guidelines for Cane Farmers on use of Amino Nitrogen Data

What is Amino Nitrogen?

Amino nitrogen is a measure of the nitrogen associated with amino acids in cane stems. Nitrogen is taken up and stored by sugarcane in the cane stem as amino nitrogen. When the growing plant requires stored nitrogen, it is transported from these amino acids to where the nitrogen is required.

Why measure Amino-N?

Amino nitrogen is measured in cane stems as supplied to the mill to provide a guide to the nitrogen status of the harvested crop. Measuring the level of nitrogen in amino acids in the cane juice (referred to as amino-N) provides this information.

Measuring amino-N is a very good method for picking up situations where nitrogen is in excess because sugarcane stores its excess nitrogen as amino-N.

How is Amino-N measured?

Amino-N is usually determined through a laboratory analysis of the cane juice called the *ninhydrin reactive test*. In a sugar mill, NIR is able to measure amino-N of the cane juice instead. Using NIR allows all rakes of cane delivered to the mill to be measured for amino-N.

What does Amino-N tell me?

Amino-N provides a guide as to the nitrogen status of the harvested crop. The Amino-N measurement is a reflection of the balance between nitrogen supplied to the crop, and that crops demand for nitrogen.

Amino nitrogen measures the balance between crop demand for nitrogen and nitrogen supply.

When amino-N is high, it is an indicator the nitrogen supplied to the crop was in excess of crop demand. When amino-N is low, it is an indicator the crops demand for nitrogen was not met sufficiently by the nitrogen supply.

The factors that influence crop demand for nitrogen and supply of nitrogen to a crop are the same factors that influence amino-N.

The Amino-N 'balance'

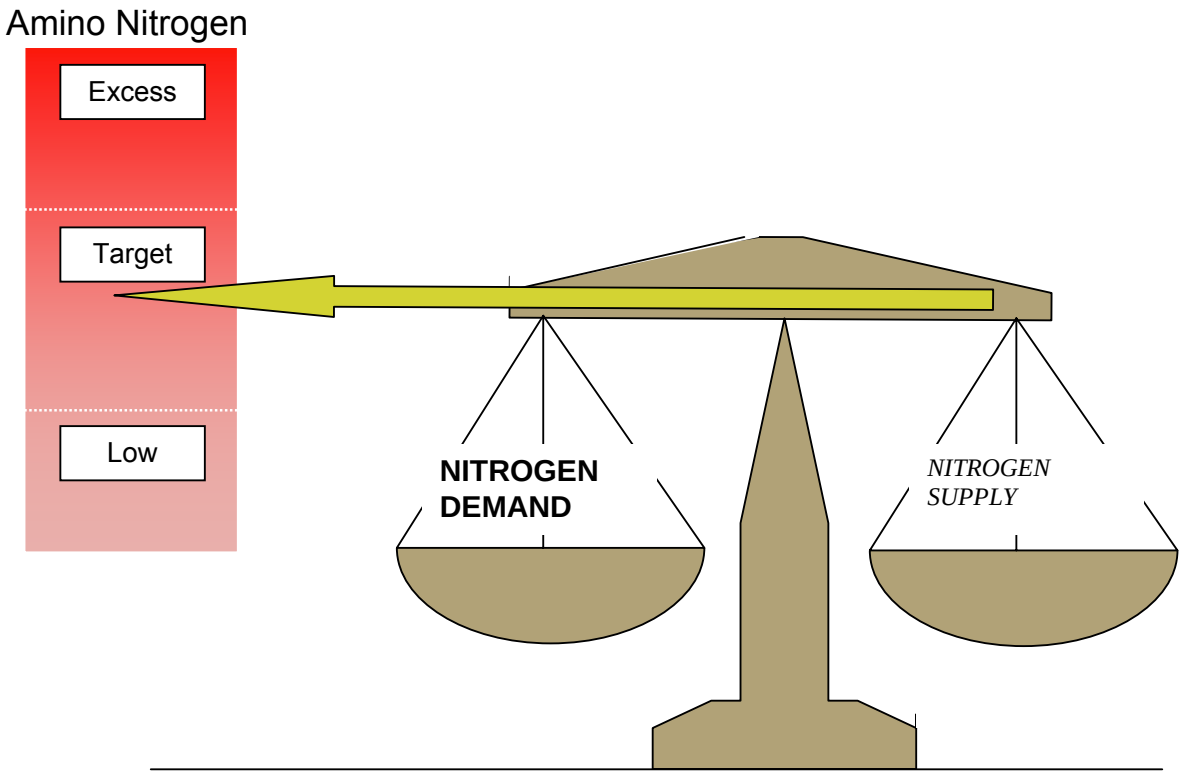
A balance can be used represent the amino-N measurement.

On one side of the balance is nitrogen supply. The greater the nitrogen supply, the further down the balance moves on this side.

On the other side is nitrogen demand. Crops with a large demand for nitrogen will cause this side of the balance to move downwards.

When crop demand and nitrogen supplied are in balance, amino-N is in the target range.

Target Range Amino Nitrogen

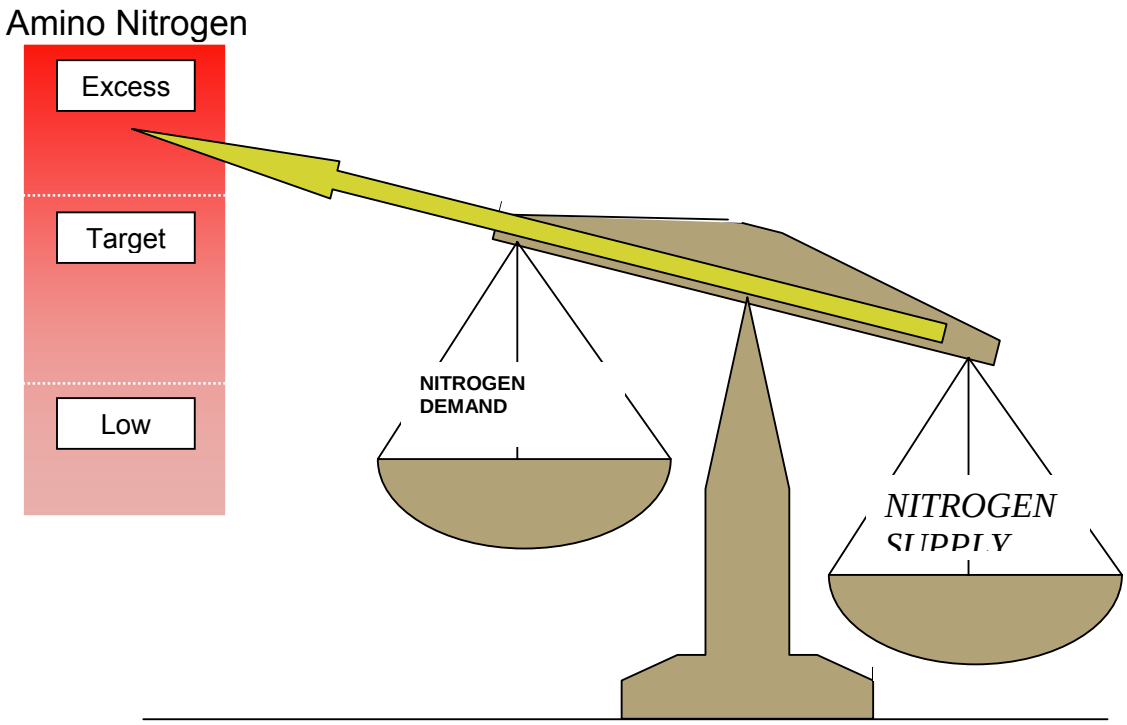


When the nitrogen demand of a crop and the nitrogen supplied to the crop are in balance, the amino nitrogen will be in the target range.

A small crop with a low nitrogen demand supplied with a low amount of nitrogen will be in balance. A large crop with a heavier nitrogen demand will also be in balance if it is supplied with more nitrogen.

Factors that influence **NITROGEN DEMAND**	Factors that influence **NITROGEN SUPPLY**
• Crop Size – weather – water logging – crop lodging – location – ratoon age – pest damage – disease incidence – other nutritional constraints • Possibly variety	• N fertiliser inputs (rates, forms, timing and methods) • Soil N sources (soil type and history) – mineral N residues – mineralisation • Mill wastes • Crop residues • Other minor N sources (rainfall, fixation) • N loss processes – leaching, denitrification, run off, volatilization

Excess Amino Nitrogen

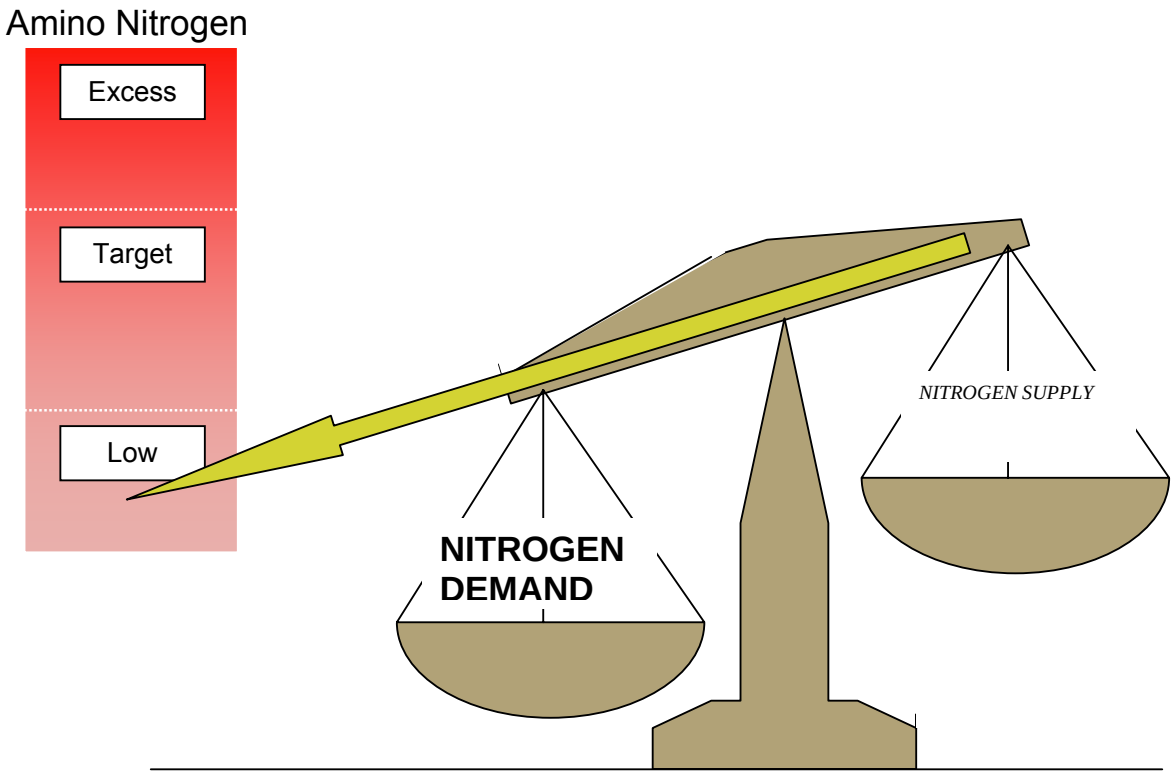


When amino-N is in the excess range it indicates nitrogen supply out-weighed nitrogen demand by the crop. This could result from growing a small crop, or over supply of nitrogen.

Excess amino nitrogen means nitrogen was wasted because there was more available than the crop needed. Excess amino-N suggests nitrogen may leave the farming system (because it is in excess), possibly contaminating surface or ground waters.

Factors that cause a light **NITROGEN DEMAND**	Factors that cause a heavy **NITROGEN SUPPLY**
• Any factor that causes small crop size: – Unfavourable weather – stool damage – water logging – crop lodging – very old ratoon – excessive pest damage – high disease incidence – other nutritional constraints • Loss of N fertiliser after application: – Leaching to ground water – Volatilisation to atmosphere – Run off to surface water – Denitrification to gases – Consumption by soil organic matter • Possible variety influences	• High N application for current crop • High residual N from high N application to previous crop • Mill mud or other wastes in addition to normal N fertiliser rate • Fallow ground prior to planting • Growing legumes prior to planting • Crop residues incorporated prior to planting • Soil N sources (influenced by soil type) – mineralisation – mineral N residues • Some minor N sources (rainfall, fixation)

Low Amino Nitrogen



Low amino-N indicates crop demand for nitrogen outweighed nitrogen supply.

Large crops have a higher demand for nitrogen than smaller crops. Low amino nitrogen will occur when not enough nitrogen is supplied to the crop, or when nitrogen is lost from the system before the crop is able to take nitrogen up.

Factors that cause a heavy **NITROGEN DEMAND**	Factors that cause a light **NITROGEN SUPPLY**
• Any factor that causes large crop size: – Favourable weather – Irrigation – Plant/Replant crops – Good stools – Adequate nutrition – Controlled pests and diseases • Possibly variety	• Low N fertiliser inputs • Applying unavailable forms of fertiliser • Incorrect fertiliser placement • N losses after application: – Leaching to ground water – Volatilisation to atmosphere – Run off to surface water – Denitrification to gases – Consumption by soil organic matter

SRDC Project CTA045 – Consultative Group meeting

"Improving CCS in the wet tropics via block-specific monitoring of N in cane delivered to the mill"

Date: Tuesday, 27 March 2001
Place: Sugar North Ltd.

Meeting opened at 2.10 am

Attendance: Brian Keating (chair), Tony Webster, Alan Hopkins, Steve Staunton, Bob Rossi, Scott Grimley, Les Robertson.

Apologies: Alan Cole, Basil Micale, Russell Muchow, Alec Ford, John Reghenzani.

Brian Keating opened the meeting by welcoming those present. The agenda was accepted.

Item 1: Juice sampling and NIR validation. Mossman and Mulgrave data was used to validate the equation produced in 1999. NIR can now recognise high values of amino-N. Comment was made on the bimodal distribution of amino-N from laboratory analyses in 2000. Of concern is the large deviation between laboratory and NIR analyses (five times higher than expected; +/- 100-200ug/ml compared to 10-20ug/ml between duplicate samples in laboratory analyses). There is no apparent correlation between NIR amino-N in prepared cane and laboratory juice analysis.

In discussion it was noted that NIR data from Green Hill generally showed higher average amino-N in cane (and also ash). Reasons for higher amino-N may include cane stress, and long-term trash blanketing.

NIR may be more appropriately used for amino-N analysis in first-expressed juice, rather than in prepared cane. The signal from prepared cane may be masked by soil and trash.

Item 2: Interactions with growers. Mossman and Mulgrave growers received reports from the project. Grower interest is high. Most collaborating growers are aware of the problem of poor correlation between NIR amino-N and the laboratory sampling.

Grower trials include changes in N-application rate of +/- 20%, and application of Dynamic Lifter and mill mud (and perhaps molasses and soybeans).

Item 3: 10th Australian Agronomy Conference. The paper presented by Brian Keating, entitled "A monitoring system based on amino-N at harvest time to improve nitrogen management in sugarcane systems" was discussed briefly. Data in the paper from

Bundaberg (obtained from trials by Ross Ridge) indicated a clear variety difference over a range of irrigation inputs, suggesting that amino-N increased with drought stress in cane. These results, and those from Alan Rattay in the Burdekin, may be confounded by absolute cane yields, and relative levels of N-demand and N-supply.

Item 4: Data analysis – amino-N and CCS. Overall, there was no correlation between NIR amino-N and CCS, either at Mossman or Mulgrave. However, when crop classes were separated, there was a negative correlation between amino-N and CCS in plant cane. The strongest correlation was for fallow-plant blocks. The relationship became weak or non-existent with increasing ratoon age. The low CCS with high amino-N in fallow-plant cane could indicate a cause-and-effect relationship that deserves further study.

ACTION. Further work is required where growers will be consulted about the history of individual blocks, to improve interpretation.

ACTION. Steve Staunton agreed that the NIR validation data needed to be revisited to examine the retationships between amino-N and crop class.

Item 5: Data analysis – amino-N and EM. Results were presented from cane grown at two N rates. Trash plus leaf had higher amino-N while neither cabbage nor soil showed elevated amino-N at higher N application rates. Trash in the cane supply may be diluting the amino-N signal in prepared cane. This could lead to high variability in readings where the EM proportion is variable. Mulgrave had an average 11% EM in 2000.

Discussion revealed that amino-N is not as readily extracted from cane as is sucrose, and a significant proportion of amino-N is recovered in later-expressed juice. Studies are needed to determine whether the proportion of amino-N remains constant in first-expressed juice over all varieties and crop classes. This is fundamental to the project. Cane does not prepare as well when EM levels are high, and this may affect the amount of amino-N present in first-expressed juice. This may explain the high variability between laboratory juice analyses and NIR amino-N from prepared cane. Earlier work by Nils Berding in 1997 was done with fibrated clean cane and filtered juice, which gave a good correlation between the amino-N values (r^2=0.91).

ACTION. Studies are needed to determine whether the proportion of amino-N remains constant in first-expressed juice over all varieties and crop classes.

Further discussion suggested that the dilution effect of EM may mask the signal strength of amino-N in prepared cane, where amino-N is about one third of the total N in cane (0.02% cf 0.06% total N).

Item 6: Progress towards the goals of CP2002. The members agreed that the project was conducted in accordance with the strategies of CP2002 to address profitability of growers, and to work with growers and other stakeholders in a participatory framework. It was agreed that demand from growers is pulling the research at a faster rate towards outputs that can be adopted.

Item 7: Plans for improving amino-N monitoring capability. Brian Keating and Tony Webster indicated that frozen juice samples had been sent to Foss and a second laboratory to assess the feasibility of measuring amino-N in first-expressed juice. The proposal was to hire a second NIR machine from Foss in May 2001 and use this to measure amino-N in first expressed juice at a mill. This would be a backup to the NIR measurement of amino-N in prepared cane. Mulgrave or Mossman were the mills most appropriate for this juice NIR, because of the grower collaboration in those areas within this project. Mossman had a slower crushing rate which is an advantage for measuring amino-N. Tully and Racecourse were also possible mills for the juice NIR due to better chute control at Tully, and a faster motherboard at Racecourse. However, Mulgrave and Mossman should also have improved computing capacity in 2001 to measure amino-N in prepared cane. Steve Staunton indicated that they had been considering fitting an on-line NIR probe into a feed pipe carrying first expressed juice.

ACTION: Steve Staunton is to advise project staff on the most appropriate mill for juice analysis using the hired NIR machine in 2001. In addition, discussions should be initiated with Ray McDowell (Mulgrave) and Alan Johnstone (Mossman) about the feasibility of using mill laboratory staff to collect the juice samples and run these through the second NIR machine.

Item 8. Plans for grower communication. The members endorsed the proposal to continue communication with Mossman and Mulgrave growers despite the lack of correlations between laboratory and NIR amino-N from the 2000 season. The project would **not** seek additional grower collaborators in 2001. The progress of automated printouts of information from the mills to growers is well advanced, with Mulgrave having an amino-N column (currently blank) on their printout.

Item 9. Other Business.

Item 10. Date and Place of next meeting:

- Date: August (date to be arranged),
- Place: at one of the collaborating mills (to be arranged).

There being no other business, Brian Keating closed the meeting at 4.45 pm.

Appendix 33: Sugarcane stem amino acid profiles

Introduction

Amino acid composition of either sugarcane leaves or sugarcane juice has been reported since the early 1900's. Parish (1956, 1964, 1965, 1967) reported on the amino acid composition of sugarcane juice and leaves, listing 23 amino acids in sugarcane juice (Parish 1965). Asparagine and glutamine were the most abundant amino acid pools, followed by aspartic acid and glutamic acid, with all other amino acids present either in very low concentrations or trace amounts (Parish 1965). Myers & Ridge (1988) reporting the influence of N fertiliser on six amino acids of sugarcane juice showed asparagine to be the major amino acid and the one most affected by the increase in applied N fertiliser. Asparagine made up 42% of the free amino acid pool when no nitrogen fertiliser was applied and increased to 61% and 63% of free amino acids when 200 and 400 kg N ha^{-1}, respectively, were applied. The other amino acids increased with the addition of fertiliser but not to the same extent as asparagine. Parish (1965) was the first to observe these dramatic increases in asparagine and total amino acid concentration with increasing application of nitrogen fertiliser. Matsui & Kitagawa (1989) concluded that asparagine acts as a storage compound being the dominant free amino acid in sugarcane stalks. Glutamine and asparagine were also the predominant amino acids in the xylem sap of sugarcane plants grown in either a glasshouse or in the field (Waldron 1976). The concentration of all amino acids measured by Waldron (1976) ranged from 70 to 1200 μg amino-N ml^{-1} sap.

Other studies have reported amino acids in sugarcane leaves (Tishchenko et al., 1991; Armas et al. 1992) with different nitrogen sources or under stress conditions (Singh & Kanwar 1992). These studies did not find asparagine to be the dominant amino acid in leaf tissue as was seen in stem juice by earlier studies. As well as cold and drought various other stress factors such as weed competition and plant infections (viral and bacterial) have been shown to affect the amino acid concentration and composition in sugarcane (Showler et al. 1990; Sastry et al. 1988; Padmanaban et al. 1988; Shukla et al. 1988). Stress factors generally resulted in an increase in the total amino acid pool.

In the pilot project (csc21S) Keating et al. showed that amino acids in the stem of sugarcane was a major pool of N and that this amino acid pool was positively correlated with stem N and sensitive to external N supply. However, the composition of the amino acid pool has not yet been reported in the series of SRDC projects of the monitoring N at the Mill.

In general, the ninhydrin reactive assay has been used in this project to determine the amino nitrogen content of sugarcane stems and juice. This assay is a rapid, cost effective bench-top assay. It does not however give a definitive concentration of all of the amino acids present and is reactive with other constituents, such as ammonium.

In this project amino acids in sugarcane samples were determined by both the bench top ninhydrin assay and by several high pressure liquid chromatography (HPLC) methods. Both the methods and the results are presented and discussed.

Methods

The ninhydrin method used is as per Keating *et al.* (1999).

High pressure Liquid Chromatography (HPLC)

Up till 1997 samples were analysed by the Department of Botany, The University of Queensland using a Beckman 6300 amino acid analyser. The analyser quantified 23 amino acids (Alanine, Arginine, Asparagine, Aspartic acid, Citruline, Cysteine, δ-Amino Butyric acid, Glutamic acid, Glutamine, Glycine, Histidine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, and Valine) and ammonium by HPLC coupled with post column ninhydrin based detection (Beckman 6300 amino acid analyzer running in physiological fluids mode). Per injection 10µl of sample was loaded in a 1:4 ratio with running buffer.

The Beckman instrument was not available after 1997 and other pre-column derivitisation HPLC methods were investigated. Initially HPLC analysis using a pre-column derivitisation with phenylisothiocyanate method based on the optimum method of Vasanitis and Molnar-Perl (1999), using an Alltech Alltima C18 column 4.6mm x 150mm. This system measured the concentration of each of 19 amino acids (Alanine, Arginine, Asparagine, Aspartic acid, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, and Valine) in cane juice. However, recovery issues with methionine, threonine, aspartic acid, proline, glycine, and glutamine meant the concentrations of these amino acids must be used with caution. An alternative method using fluorescence detection HPLC is currently being investigated.

Results and Discussion

The direct measurement of amino acids in juice by HPLC allows for the direct measurement of amino nitrogen concentration. This allows the construction of an equation to convert ninhydrin reactive N values to juice amino-N concentrations (Keating et al. 1999). The equation presented by Keating et. al. (1999) was developed in 1996 and represents the best fit from numerous ninhydrin versus HPLC comparisons (Fig 1).

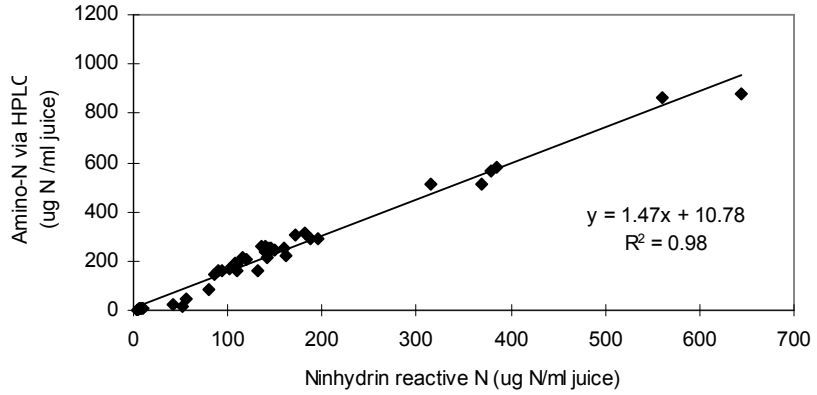


Fig 1 Relationship between ninhydrin reactive N and amino-N determined by HPLC. Relationship derived from Jeffco/Carver extracted samples from a range of N-rate trials conducted during 1994 to 1996.

To further confirm this relationship for field cane supplied to mills in north Queensland further amino acid by ninhydrin comparisons were made. Issues with the HPLC determination of juice amino acid profiles and direct measurement of juice amino-N were raised by the apparent change in the relationship of a direct comparison between HPLC amino acid and ninhydrin reactive amino acid. When expressed as a molarity the two measurements should give a 1:1 relationship. Some variation would be expected due to the imprecise nature of the ninhydrin reaction. In 1995 it was found that different data sets gave different relationships, often with the HPLC.

Profiles of the amino acids present in the samples collected allows for insight into the changes in individual amino acids that can give information on the transport, storage, and metabolism occurring in the sugarcane plant. As with many previous studies on the amino acid composition of sugarcane juice, asparagine was shown to be the dominant amino acid present (Fig 2). This further supports that asparagine is involved in N storage with in the cane stem.

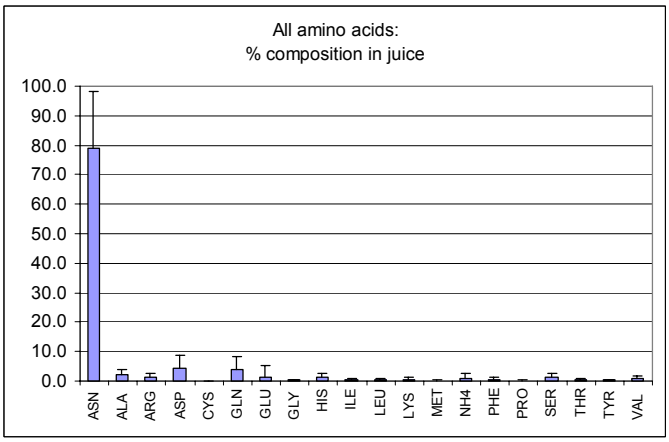


Fig 2 Amino acid profiles of samples analysed using Beckman 6300 analyser. Samples were collected in 1997 either from mills in the Bundaberg region (first expressed juice) or by Jeffco/Carver extraction from various N-rate trials.

Other amino acids present were glutamine and aspartic acid, both essential amino acids in the nitrogen assimilation, storage, transport, and metabolism. The patterns observed are similar to those found in samples collected for investigation of EM on juice amino acid composition and concentration (see Appendix 34). The EM study also used the post column ninhydrin reaction method.

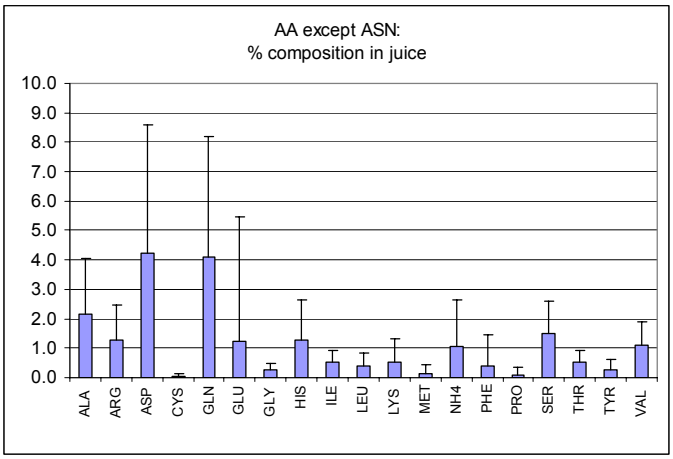


Fig 3 Amino acid profile from Fig 2 with asparagine peak removed.

Similar results were found with the pre-column derivitisation HPLC method (Fig 4). However, the relative size of the asparagine peak, relative to total amino acid concentration, is much reduced. The other interesting feature of these data is the increase in the 1 N amino acids with increasing total amino acid pool size (Fig 5).

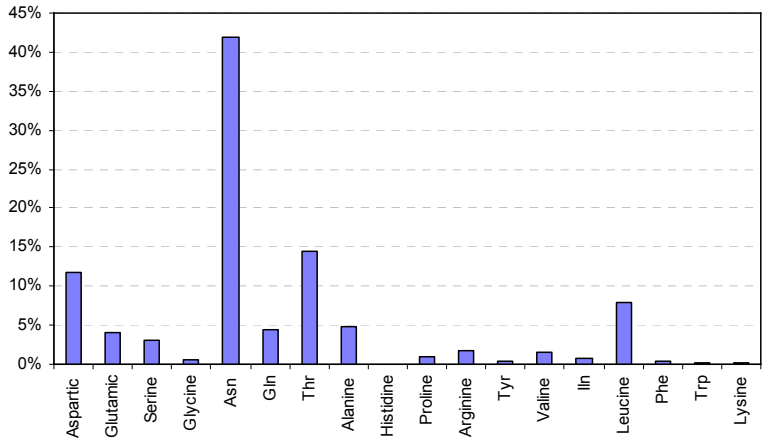


Fig 4 Profile of amino acids as determined by pre-column derivatised HPLC method.

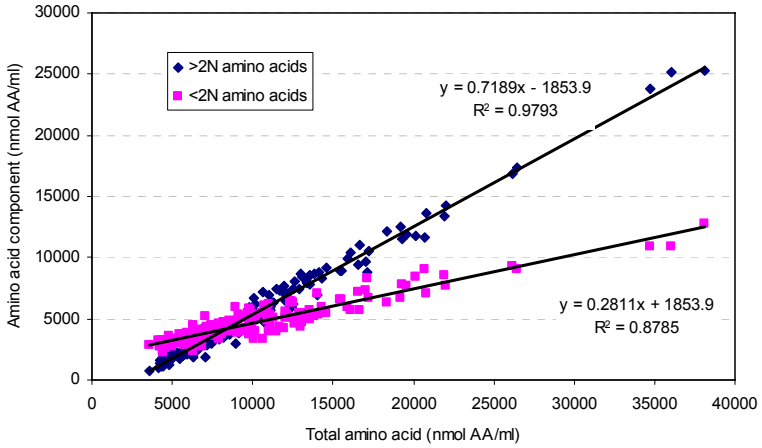


Fig 5 Change in the pool size of 1N amino acids and ≥2N amino acids with increasing total amino acid pool.

Such differences in the results have led the project team to investigate other HPLC methods for the determination of amino acid profiles to check for potential errors. The method of Vasanitis and Molnar-Perl (1999) has introduced some recovery problems that may be contributing to the different profiles and response of the amino acid pools to increasing total amino acid pool size. These results will be reported on when they are available.

Analytical procedures to overcome the recovery problems are currently being investigated. It is hoped that a fluorescence detection method will allow for better peak separation and improved recovery of amino acids in samples. A follow up report will be required to deliver these outcomes.

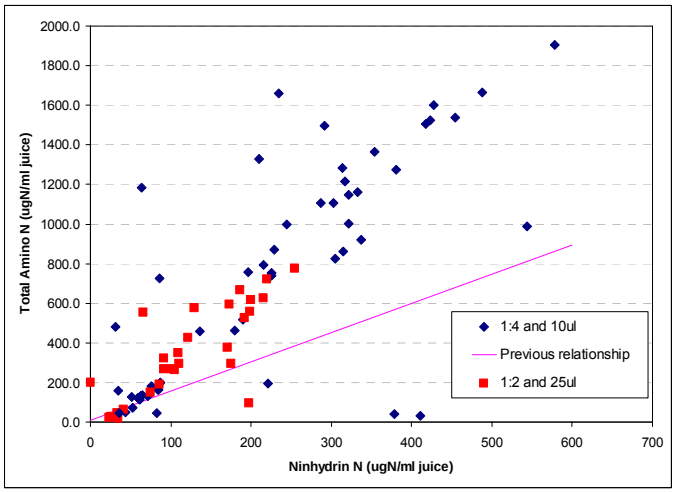


Fig 6 HPLC amino acid versus Ninhydrin amino acid for results from 1997.

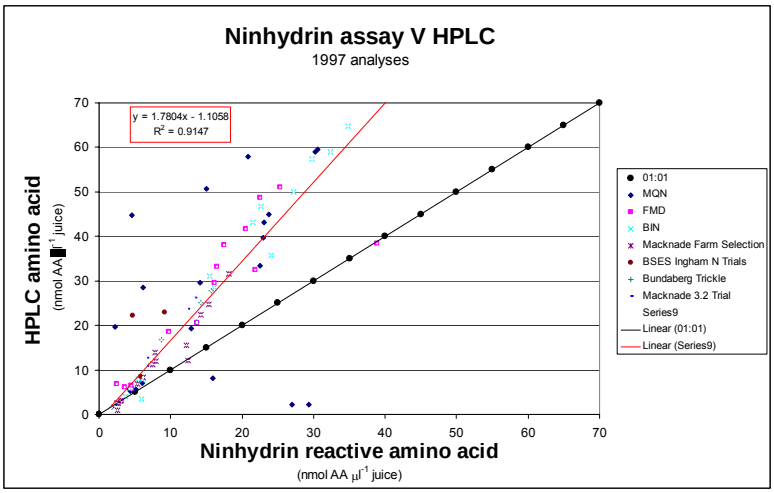


Fig 7 Comparison of amino acid pool size measured by HPLC and Ninhydrin reactive N.

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Appendix 34: Influence of extraneous matter on extraction of sugarcane juice and amino acid concentration

Introduction

Extraneous matter (EM) in sugarcane has adverse affects on many milling operations. These include, increased equipment maintenance, combustion problems, reduced operational efficiency and reductions in sugar extraction due to roller wear (Kroes & Forsell 1999). Reduced yields in the wet tropics have been partly blamed on increased levels of EM in the cane supply (Berding et al. 2002).

Are these levels of EM influencing the extraction efficiency of the juice extraction by the N^{o}.1 mill and therefore influencing the calibration of NIR scanned on the prepared cane and calibrated on the amino-N content of first expressed juice (FEJ)? Surprisingly there appears to be little literature investigating direct measurement of any reduction in juice extraction due to the presence of EM in the cane supply.

This experiment was initially conducted to investigate if EM influenced the amino-N concentration and composition of extracted sugarcane juice. It has been further used to investigate if EM influences the efficiency of juice extraction. It was hypothesised that the inclusion of EM would reduce the compressive force of the hydraulic press or milling train rollers.

The impact of EM on juice constituents was investigated using Jeffco/Carver juice extraction methods and 2 different rates on N application.

Methods

For the overall experimental layout and treatment application see Dart et al (2000).

This EM investigation was carried out in September 1997 at the final harvest for the plant crop. The 0 and 180 kg N ha^{-1} treatments were sampled but only samples from replicates 1 and 2 were analysed due to replicate 3 not growing well in the plant crop. Hand sampling of stalks from plots were conducted as presented in Dart et al. (2000). In addition, 1 to 1.5 kg samples of surface trash, green leaf, cabbage, and surface soil were collected from the treatment plots. Samples were transported to Brisbane for sample preparation and juice extraction.

For juice extraction all collected samples were ground through Jeffco cutter grinder. Juice was extraction was by Carver hydraulic press based on methods outlined in Muchow et al. (1993).

Extraneous matter was added at either 2% or 5% by weight in conjunction with millable stalk, no multiple additions were made. Each of the trash, green leaf, cabbage or soil was added at the two rates with 1kg of millable stalk and the combined sample well mixed. Due to the low density of the trash material it was not possible to pack 500g of mixed sample into the press. For 2% EM addition a 340g sample was used and for 5% EM addition a 230g sample was used. The pre and post pressing weights for the fibre were recorded to give an indication of the extraction efficiency.

The collected juice samples were analysed for amino-N by both ninhydrin assay and HPLC according to the methods set out in Keating et al. (1999). Juice samples were also analysed for total N by the Kjeldahl method.

Due to only 2 replicates being used, data is represented as the mean of the 2 replicates with maximum and minimum values also shown to give an indication of variation.

Results and Discussion

The plant crop did not show any significant differences for juice amino-N for the range of N rates (Fig 1). This lack of an N response is typical for a plant crop where there is often sufficient N in the soil to grow the crop.

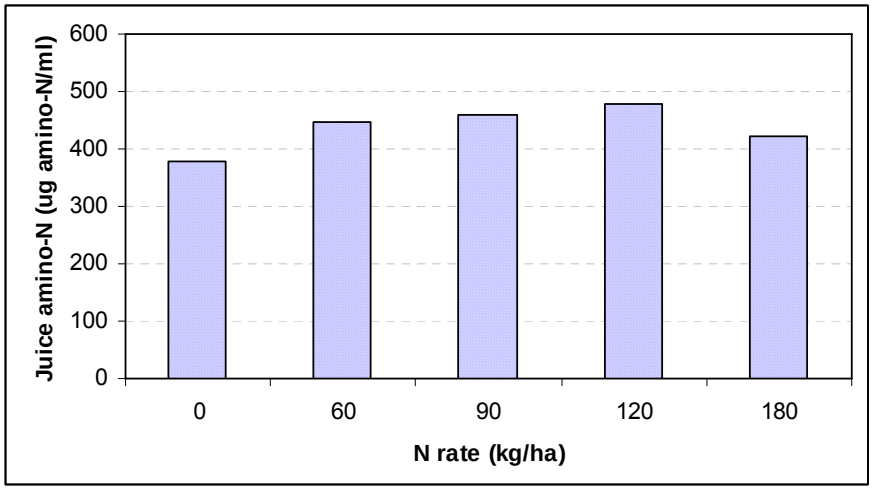

Fig 1 Juice amino-N results for end of season harvest of plant crop, September 1997.

This lack of an N response is also shown in the concentration of amino acid N after the addition of extraneous matter (Fig 2).

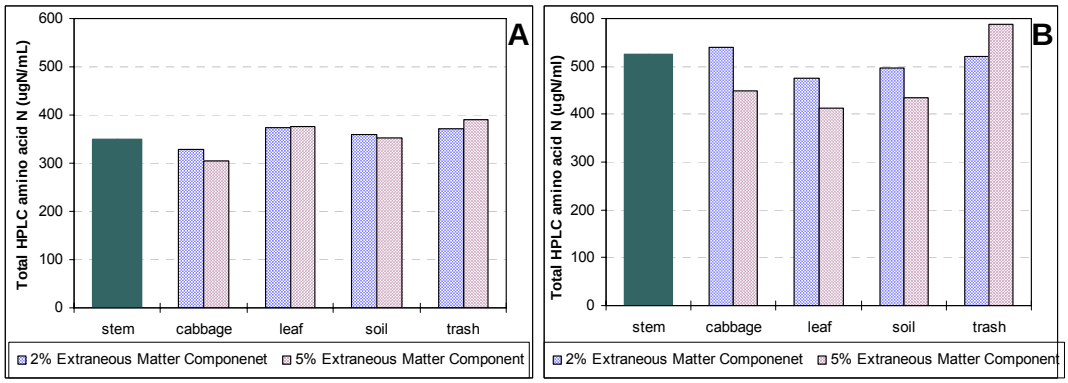

Fig 2 The concentration of amino acid N as determined by HPLC for each the EM additions. Two N rate treatments are 0 kg N ha^{-1} (A) and 180 kg N ha^{-1} (B).

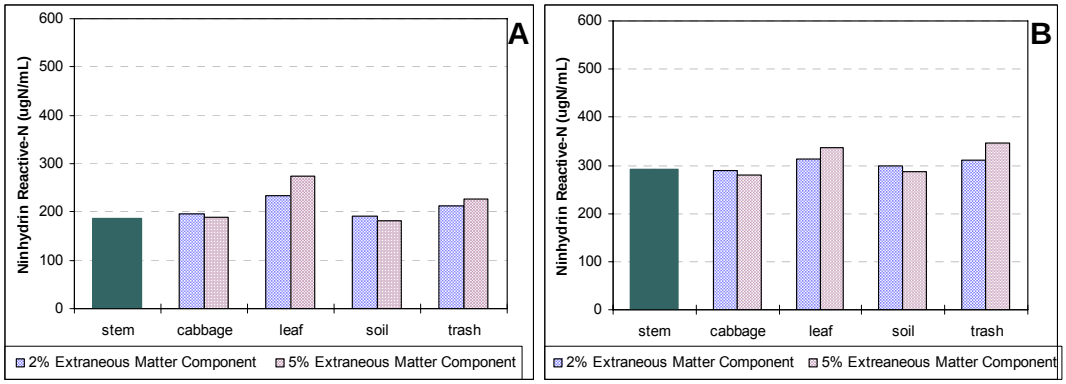

Fig 3 The concentration of amino N as determined by ninhydrin assay for each of the EM additions. Two N rate treatments are 0 kg N ha^{-1} (A) and 180 kg N ha^{-1} (B).

The overall variation in the juice amino-N values in the three figures 1, 2, and 3 are possibly due to the nature of the sampling process as well as the assay used to measure amino acids (HPLC versus ninhydrin). All samples were hand sampled and the intrinsic variation in sugarcane within a cane plot makes such sampling highly variable. The lack of a change in the concentration of amino-N per ml extracted juice due to the addition of EM is not unexpected. The EM additions would be expected to add little amino-N to the sample and the slight increases in the concentration are probably artefacts of the extraction. The EM material could be absorbing some of the water in the extracted juice for example. This is highlighted in figure 4, where the efficiency of juice extraction is illustrated. It can be seen that any addition of EM reduces the amount of juice extracted. The addition of trash EM causes the greatest reduction in juice extraction and the addition of as little as 5% trash resulted in 50% less juice extracted.

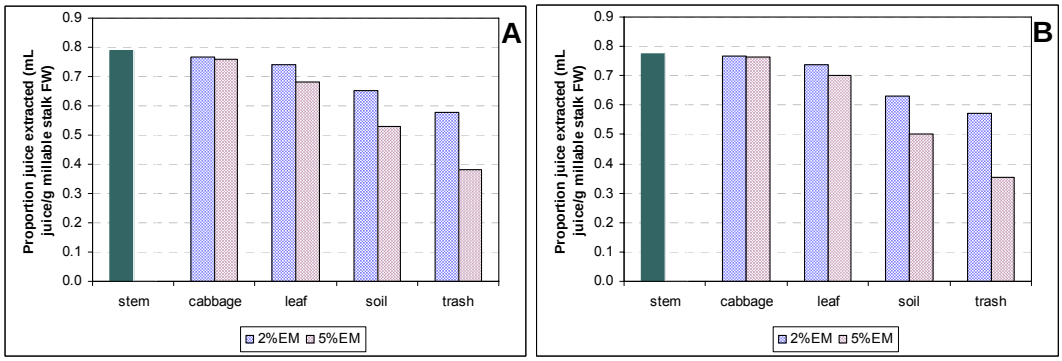

Fig 4 Juice extraction efficiency as affected by the addition of extraneous matter (EM) of different types. Stem has no EM added. Two N rate treatments are 0 kg N ha^{-1} (A) and 180 kg N ha^{-1} (B).

The results in figure 4 are somewhat surprising. This data shows the addition of 50g of soil to 1000g of millable stalk results in a 36% reduction in the amount of juice extracted and the addition of 50g of cane trash resulted in reduction of 54%. Such high juice extraction reductions would have dramatic consequences in a mill where the level of EM is often higher than 5%. The degree of pressure used to extract juice was shown to not have an impact in

an experiment to investigate the effect of cane preparation and hydraulic pressure on extraction of amino-N.

The size of these extraction reductions seen in this experiment must be due to the interaction of the EM and the nature of the carver press method compared to the milling train of a commercial mill. This reduction in juice extraction results in increased concentration values for amino acids (Fig 5). This is especially evident with the 5% trash EM. The trash may be absorbing water from the juice hence concentrating the juice solutes.

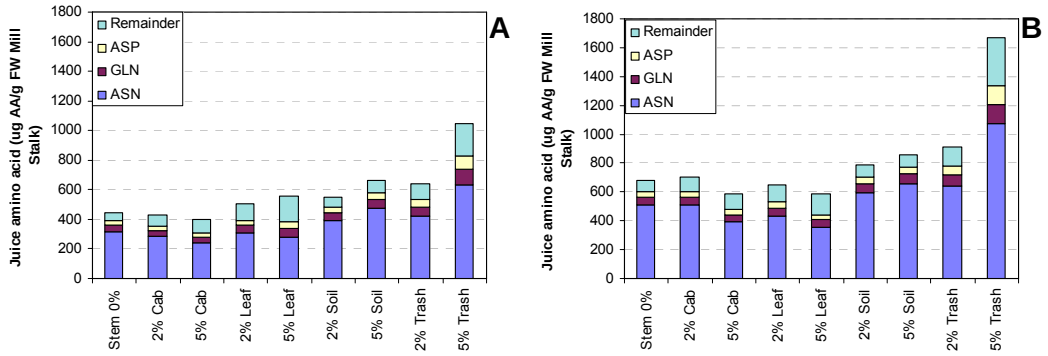

Fig 5 Amino acid concentration for juice extracted after the addition of extraneous matter from 2 N rate treatments (A = 0 kg N ha^{-1} and 180 kg N ha^{-1}).

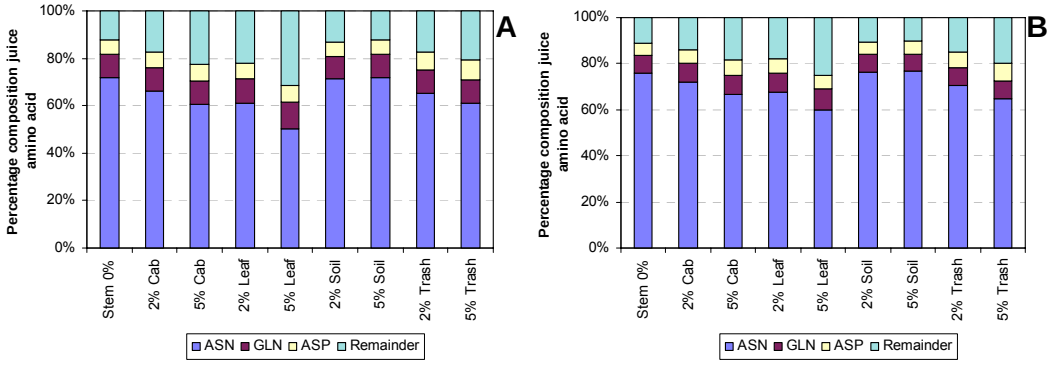

Fig 6 Relative amino acid composition for juice extracted after the addition of extraneous matter fro 2 N rate treatments (A = 0 kg N ha-1 and 180 kg N ha^{-1}). Only asparagine

Conclusion

From this study the evidence would show that there is a dramatic change in the amino-N composition of the extracted juice and the amount of juice extracted when EM is added. Personal communications would suggest that this is not the case in the mill.

More studies are required to investigate the relationship of amino-N and EM at a mill scale. This will potentially require collection of prepared cane and bagasse after the N^{o}.1 mill.

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