Assessment of leaf analyses of sugarcane under moisture stress conditions

by

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Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Agricultural Sciences, University of Pretoria.

Brisbane, Australia April, 2000

Contents

Declaration

I hereby declare that the material presented in this thesis is based on my own original work, except where otherwise acknowledged, and that it has not been submitted previously for examination at this or any other university.

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Acknowledgments

I would sincerely like to thank:

- Prof. Robin Barnard, my supervisor, for his continued support with this project and especially in more recent times when his own circumstances had changed considerably;
- Dr. Peter Hewitt and Mr Roger Bond, Director and Asst. Director respectively of the South African Sugar Association Experiment Station (SASEX), for allowing me to undertake much of this study during my employment at Mt Edgecombe;
- Dr. Colin Ryan, Chief Executive Officer of the Bureau of Sugar Experiment Stations (BSES), and Prof. Robert Lawn, Director of the Cooperative Research Centre for Sustainable Sugar Production (CRC-SSP) for the opportunity to complete this study in Australia;
- Mr. Jan Meyer, Head: Soils and Nutrition at SASEX for his input and continued interest in this project;
- Dr. Peter Twine, Group Manager: Agronomy and Crop Protection, and Dr. Graham Kingston, Principle Research Officer (BSES) and Program Leader (CRC-SSP) for encouraging and facilitating the completion of this dissertation:
- Miss Joanne Stringer, Biometrician (BSES) for her advice and assistance with the statistical analysis of some of the data;
- Dr. Geoff Inman-Bamber, former Principal Agronomist at SASEX, for fulfilling the role of co-supervisor during the initial stages of this work;
- Dr. Andrew Wood, Chief Technical Field Officer (CSR Sugar (Ltd)) for reviewing the manuscript;
- Mssrs. Suran Moodley, Lee Aitken and Tim Shapter for their technical assistance during various stages of the experimental work;
- Miss Angira Rampersad and staff of the SASEX fertiliser advisory service laboratory for the preparation and analysis of the large number of samples associated with the initial trials in this study;
- Mrs Zofia Ostatek-Boczynski (BSES) for the chemical analysis of the leaf samples associated with some of the latter trial work;

- Drs. Robert Aitken and Philip Moody, Principal Soil Scientists (Queensland Department of Natural Resources) for allowing me to use the glasshouse facilities at Indooroopilly for the final stages of this investigation;
- Cecilia Schroeder, my mother, and parents-in-law Mora Wood and, John and Veroni Wood, for their continued interest in my work and their on-going encouragement for me to complete this project;
- and lastly, but by no means least of all, my wife Shona and children Kevin and Kate, for their patience, loyalty and understanding during the long years of this study. Without your support this thesis would not have been possible.

I would like to dedicate this dissertation to my father Gordon Schroeder (4 July 1921 – 2 July 1993), who saw the beginning of this work but not its completion. "Dad, my sincere thanks for your love and the sacrifices you made in helping to provide the means for me to have a university education."

Abstract

Leaf analysis continues to be an important 'tool' used for diagnostic and advisory purposes in a number of world sugar producing industries. This has especially been the case in countries such as South Africa where leaf analysis is routinely used to check on the adequacy of fertiliser recommendations and as a means to assess nutrient trends in the various regions and the sugar industry as a whole. In contrast, following limited use of leaf testing in the Australian industry, there has recently been a resurgence in interest in leaf sampling as a means of facilitating better nutrient management. Despite the level of historical usage, a set of general third leaf critical values that covers most of the essential plant nutrients was developed by various research scientists in the various sugar producing countries over the years, with slight modifications based on local conditions and experience. Whether solely used for diagnostic purposes, or for more advanced interpretation of nutrient trends or interactions, it is known that leaf analysis data can be affected by various factors such as crop age, season, variety and the presence of moisture stress. Guidelines and prerequisites associated with leaf sampling, and an understanding of the effect of these factors on leaf nutrient values, form an essential component of the overall concept of leaf analysis. Despite recognition that plant water relations may markedly influence plant nutrition, little quantitative information is available regarding the effect of moisture stress on the nutrient content of sugarcane.

The work reported in this thesis was aimed at assessing leaf analysis and the macro nutrient content of young sugarcane under conditions of moisture stress, by

i. Reviewing the development and use of leaf analysis with sugarcane
ii. Assessing evidence from the South African sugar industry that moisture stress was affecting leaf analysis data
iii. Investigating the interaction between moisture stress and the macro nutrient content of sugarcane at an age when leaf sampling is normally practiced
iv. Comparing the interaction of moisture stress and the nutrient content of three different sugarcane varieties
v. Establishing a moisture stress indicator for improved interpretation of sugarcane leaf analysis data.

The project consisted of four distinct, but inter-related, phases. The initial phase centred around i) and ii) above. In the case of ii), examples of data were considered where commercial sugarcane fields had been leaf sampled during and subsequent to moisture stress conditions, as were mean third leaf nutrient values pertaining to selected regions over a range of 'normal' and 'drought-affected' seasons. In addition two case studies were conducted to assess the affect of moisture stress on leaf nutrient values on a whole-farm basis.

The second and third phases of the project (aims iii. and iv.) were conducted in semi-controlled conditions (under an automatic rain-shelter or within a glasshouse). In phase two, the interaction between moisture stress, plant growth and plant nitrogen content was assessed in two experiments in which sugarcane was grown in large pots (80 litre) within a 4 x 4 (moisture stress x sampling date) factorial trial in which N was adequately supplied (Trial 1), and in a 2 x 2 x 4 (N application rate x moisture stress treatments X sampling date) randomised pot trial where two rates of N (equivalent of 120 kg N ha^{-1} and 60 kg N ha^{-1}))were considered. In Trial 1, the moisture stress treatments were as follows: unstressed - soil kept at field moisture capacity; stressed (early) - water withheld from day 90 after planting; stressed (late) - water withheld from day 100 after planting; and stress/relief - water withheld from day 90 after planting, but soil rewatered to field capacity on day 110 after planting. The four harvest (complete destructive sampling) dates were separated by 10 days and began on day 100 after planting. In Trial 2, only two moisture stress treatments were considered ie. unstressed (as above) and stress/relief – water was withheld from day 140 after planting, but soil rewatered from day 165 after planting. The four sampling dates were again separated by 10 days that began on day 145 after planting. These trials were also used to assess the interaction between the other leaf macro-nutrients (P and K) and moisture stress.

In the third phase, two concurrent trials were conducted (Trial 1(Qld) and Trial 2 (Qld) to assess the interaction between the macro-nutrients (N, P and K) and moisture stress in three sugarcane varieties (NCo310, Q141 and Q136). In this case, sugarcane was grown in 40 litre pots within the 2 x 2 x 3 (variety x moisture stress x sampling date) randomised pot trials. The two moisture stress treatments were as follows: unstressed (as above) and stress/relief (water was withheld from day 100 after

planting, but stress relieved by rewatering from day 110 after planting). Sampling was conducted on three dates beginning on day 120 after planting.

The final phase consisted of an investigation to establish a robust moisture stress indicator that could be used to identify moisture stress at the time of sampling, and provide a means of assessing or interpreting leaf analysis data (particularly N) under such conditions.

The examples and case studies used to assess evidence of a moisture stress effect in the industry showed that drought effects associated with below 'normal' rainfall had indeed influenced the nutrient content of the third leaf samples. The occurrence of abnormally large numbers of low leaf N and P values were the result of moisture stress effects rather than nutrient deficiencies *per se*.

As expected, plant extension rate, leaf area index (LAI) and dry matter production were all negatively affected by the imposition of moisture stress over the sampling periods in all of the trials. Significant increases in these parameters occurred with stress relief. In relation to plant N, it was found that there was a significant interaction between moisture stress treatment and sampling date. Compared to the unstressed sugarcane, the total plant N declined markedly with imposition of moisture stress (when N was adequately applied), but improved considerably with stress relief, resulting in no significant differences between the unstressed and stress/relief sugarcane on the last sampling date. These differences in plant N, due to moisture stress effects, were also generally apparent when the harvested plants were partitioned into their component parts (spindle, leaf and sheath number and trash). In particular, it was found that the moisture stress treatments and date of sampling had a significant effect on third leaf N content. However, when N was limiting, little recovery in total and third leaf N was apparent once stress was relieved.

In partitioning the plants, it was found that like N, the plant P and K concentrations declined with increasing leaf and sheath numbers. Generally, total and third leaf P value were less sensitive than plant N to moisture stress effects. Plant K was generally found to be insensitive to moisture stress. In terms of the third leaf nutrient values (N, P and K) there was no evidence of varietal differences between the three varieties

(NCo310, Q141 and Q136) under either unstressed or stress/relief conditions. Trends in leaf N, P and K grown in sub-optimal (yet balanced) nutrient conditions were similar to those observed when nutrients were adequately supplied.

It was found that the dry mass of the top sections of the third leaf laminae (between the 200mm section (used for chemical analysis) and the leaf tip) expressed as a percentage of their wet mass (D%W(L3T)) used in combination with the dry mass of a sample of spindles (from the same plants) expressed as a percentage of their wet mass (D%W (Sp)), provided a useful indicator of moisture stress in sugarcane at the time of leaf sampling. D%W (L3T) values of less that 32% in combination with D%W (Sp) values less than 22% would indicate unstressed conditions in sugarcane at the time of sampling. D%W (L3T) values greater than 32% in combination with D%W (Sp) values above 22% would indicate stressed conditions. D%W (L3T) values above 32% in combination with D%W (Sp) less than 22% would indicate stress-relieved conditions but with inadequate recovery of the third leaves (moisture and nutrients). In cases where D%W (L3T) indicated moisture stress conditions, estimation of 'unstressed' third leaf N values corresponding to third leaf N values affected by moisture stress (as quantified by a D%W (L3T) value) was found to be possible, using a regression equation (r^2=0.656) that linked relative third leaf N values (actual third leaf N values expressed as a percentage of baseline values) to D%W(L3T). Although the estimation of 'unstressed' third leaf P values corresponding to third leaf P values affected by moisture stress was also found to be possible, the appropriate regression equation was weaker than that associated with the third leaf N values.

In general, it was concluded that total and third leaf N, P and K values are differentially affected by moisture stress and stress/relief conditions. The decline and recovery in plant N values with time when water was withheld and then re-applied confirmed the interaction between water availability and the N content of sugarcane. However, due to the work reported in this thesis, this interaction has now been quantified and is more fully understood. The proposed use of D%W (L3T) and D%W (Sp), together with the regression equations relating D%W (L3T) to relative third leaf nutrient values provides a useful remedy for dealing with moisture stress conditions during leaf sampling. The substantially eased constraints on leaf sampling will

hopefully encourage renewed, and possibly greater, use of leaf analysis for better nutrient management in sugarcane production.

General introduction

Leaf analysis is a nutritional 'tool' that is successfully used in a number of agricultural industries around the world. In particular, the sugar industries of countries such as South Africa, Brazil and Mauritius have recognised the value of foliar testing as a means of better managing and targeting nutrient inputs. Growers in the South African sugar industry, for instance, are encouraged to regularly leaf sample their ratoon sugarcane crops, as a means of checking on the adequacy of fertiliser recommendations based on soil samples collected prior to the establishment of plant cane. In other countries, such as Australia, there has been a resurgence in interest in leaf analysis as a means of managing and/or monitoring nutrients in sugarcane, particularly due to a perceived over-application of N and P, and possible under-application of K. Irrespective of the level of utilisation of leaf analysis in the different sugar industries, much effort has over the years been directed towards establishing, developing and/or confirming suitable critical (or threshold) values for use with sugarcane.

However, one specific aspect of leaf analysis that has consistently been problematic is the effect of drought stress on nutrient concentrations in plant tissue. Although it has long been recognised that moisture stress affects the nutrient content of sugarcane leaves, there is no evidence to suggest that this effect has been comprehensively investigated under controlled conditions. As such, little quantitative data is available on which to base interpretation guidelines for dealing with leaf analysis data affected by moisture stress, although some attempts have been made to use surrogates, such as nutrient ratios, for this purpose. In addition, the absence of a simple yet robust moisture stress index for use with leaf analysis in sugarcane has continued to hamper the meaningful interpretation of leaf analysis data.

In view of the above, this investigation was aimed at assessing leaf analysis as a tool for continued use in sugarcane production and then to assess the major nutrient content of sugarcane under moisture stress conditions. This would ultimately enable

the development of suitable guidelines and a moisture stress indicator to ensure better interpretation of leaf analysis data that may be affected by moisture stress.

Chapter 1.

Leaf analysis: A review of its development and use in sugarcane production

1.1. Introduction.

Leaf analysis has, and continues to be, successfully used as a nutrient diagnostic and advisory tool in many cropping industries throughout the world. The fact that leaf samples are analysed to determine the nutrient content of a crop is based on the work of the early chemists such as Liebig (1840), Hall (1905), and Mitscherlich (1909), who recognised that relationships existed between crop yield and the nutrient content of plant ash. By establishing methods for interpreting these relationships, Macy (1936) provided a platform for using plant tissue testing as a means of assessing the nutrient status of a crop (Smith and Loneragan, 1997). As a result, many resources have over the years been devoted to gathering data and establishing critical leaf values for various horticultural and field crops worldwide. This work has resulted in leaf analysis being used for three distinct applications in the overall effort of achieving better nutrient management in various crops:

- Diagnosis of existing problems (nutrient deficiencies, toxicities and/or nutrient imbalances;
- Prediction of nutrient deficiencies in current (likely between sampling and harvest) or succeeding crops;
- Monitoring the crop nutrient status (effectiveness of fertiliser practice, crop removal, overall nutrient status of regions, districts, soil type, etc)

(Smith, 1986; Smith and Loneragan, 1997).

Sugarcane has by no means been an exception in this regard. However, it is apparent that although a continually evolving system of leaf analysis is ultimately of benefit to all world sugar producers, some countries have been more active than others in its development and use. For instance, while the use and development of leaf analysis has actively been pursued in the South African (Schroeder *et al*, 1992), Brazilian (Malavolta, 1994) and Mauritian (Ng Kee Kwong and Deville, 1983) sugar industries, there is currently limited use of leaf testing in the Australian industry (Schroeder *et al*, 1998). These differences in interest have resulted

in various levels of sophistication in the use of leaf testing amongst the world sugar industries. At one end of the scale is a somewhat rudimentary system consisting solely of general critical leaf values that are most often used for diagnostic purposes. At the opposite end of the range is an evolutionary system where there is continuing confirmation and refining of critical values and methods (or tools) for more effective interpretation of leaf analysis data. With a more developed system there is more scope for using leaf analysis data for a combination of diagnostic, advisory and nutrient trend purposes (Schroeder *et al*, 1992; Schroeder *et al*, 1993). However, it is considered essential that at each level, the leaf analysis norms and prerequisites for sampling are well defined and based on sound scientific principles.

1.2. Critical (threshold) values for diagnostic purposes

In the development of leaf analysis for diagnostic purposes, a range of leaf nutrient values has been established and categorised according to the terms marginal, critical and adequate for cane production. Traditionally, these values relate to the middle 300mm section of the lamina associated with the top visible dewlap (TVD) of the sugar cane plant, which normally corresponds to the third leaf below the spindle (Clements and Ghotb, 1968). Although a comprehensive list of these values was collated by Reuter and Robinson (1997), only the third leaf critical values from four world sugar industries (Australia, South Africa, Mauritius and Guyana) are presented in Tables 1.1 and 1.2. These cover the macro and secondary nutrients, and some micronutrients or trace elements. It is generally apparent that the third leaf critical values used in the four industries are not dissimilar to each. The differences that do exist, however, appear to be due to either variations in recommended sampling ages or the result of fine-tuning of the established critical value for particular circumstances. The critical values that have been established in the Australian and Mauritian sugar industries refer to samples that need to be collected when the cane is 2-4 and 5 months old respectively. In South Africa and Guyana, the period for sampling has been extended by establishing modified critical values based on crop age. This allows recognition of the fact that N values, in particular, decline with age and time of season. Recognition of varietal differences has also resulted in some 'fine-tuning' of critical values in the South African industry.

Table 1.1. Third leaf critical values for macro and secondary nutrients in sugarcane.

Nutrient	Third (or top visible dewlap) leaf critical values (%)									
	Australia[a]	South Africa[b]					Mauritius[c]	Guyana[d]		
N	1.8 (3 mnths)	**Area**	**Crop age (mnths)**	**Month of sampling**	**P[e]**	**R[f]**	1.95 (5 mnths)	**Crop age (mnths)**	**P[e]**	**R[f]**
		North	3 – 5	Oct-Dec Jan-Feb Mar-Apr	1.9 1.8 1.7	1.8 1.7 1.6		2 3 4.5 5 6	 2.4 – 2.5 2.1 1.9	2.4 – 2.5 2.1 1.9
		Coastal	4 – 7	Nov-Dec Jan-Feb Mar	1.9 1.8 1.7	1.8 1.7 1.6				
		Midlands	4 – 9	Nov-Dec Jan-Feb Mar	1.9 1.8 1.7	1.8 1.7 1.6				
P	0.19 (3-4 mnths)	**Variety**		**Areas & crop ages as shown for N**			0.21 (5 mnths, ratoon)	**Crop age (mnths)**	**P**	**R**
		N12 Other N & NCo varieties		0.16 0.19				2 3 4.5 6	 0.21 0.18	0.21 0.18
K	1.1 (3-4 mnths)	**Variety**	**Harvest season**	**Month of sampling**	**Areas & crop ages as shown for N**		1.25 (5 mnths, ratoon)	**Crop age (mnths)**	**P**	**R**
		N14	Winter (irrigated crop)	Oct-Nov Dec-Jan Feb-Apr	0.70 0.80 0.90			3-6 2-4.5	1.25	1.250
			Other	Oct-Apr	0.90					
		All other N & NCo varieties	Winter (irrigated crop)	Oct-Nov Dec-Jan Feb-Apr	0.85 0.95 1.05					
			Other		1.05					
Ca	0.2 (3-4 mnths)	0.15 (areas and crop ages as shown for N)					0.20 (5 mnths)	0.13-0.15 (3 mnths)		
Mg	0.08 (3-4 mnths)	0.08 (areas and crop ages as shown for N)					0.10 (5 mnths)	0.08 (rapid growth)		
S	0.13 (3mnths) S low if N:S>17	0.12 (areas and crop ages as shown for N)					-	-		
Si	0.7 (3-4 mnths)	-					0.7 (5 mnths	-		

[a] Calcino,1994; [b]Schroeder *et al*,1992 or Meyer *et al*.,1971; [c]Bassereau, 1988 or Halais, 1962; [d]Evans, 1965
[e] Plant or replant; [f] Ratoon cane.

Table 1.2. Third leaf critical values for micro nutrients in sugarcane.

Nutrient	Third (or top visible dewlap) leaf critical values ($mg\ kg^{-1}$)			
	Australia[a]	South Africa[b]	Mauritius[c]	Guyana[d]
Cu	2 (3-4 mnths)	3 (areas and crop ages as shown for N)	5 (5 mnths)	3.5 (rapid growth)
Zn	10 (3-4 mnths)	15 (areas and crop ages as shown for N)	20 (5 mnths)	15 (rapid growth)
Mn	15 (3-4 mnths)	15 (areas and crop ages as shown for N)	15 (5mnths)	15 (rapid growth)
B	1 (3-4 mnths)	1 (areas and crop ages as shown for N)	1 (5 mnths)	1 (rapid growth)
Mo	0.08 (3-4 mnths)	-	0.1 (5 mnths)	0.08 (rapid growth)

[a] Calcino,1994; [b]Schroeder *et al*,1992 or Meyer *et al*.,1971; [c]Bassereau, 1988 or Halais, 1962; [d]Evans, 1965

1.3. Leaf sampling and factors influencing leaf analysis

Leaf analysis in combination with soil testing is considered a very useful method for determining balanced nutritional programmes for sugarcane. While soil analysis procedures estimate the amount of plant available nutrients, leaf analysis reflects the actual plant nutrient uptake until the sampling date (Smith and Loneragan, 1997). However, it has long been recognised that a number of factors can influence plant nutrient uptake and therefore the nutrient content of leaves (Gosnell and Long, 1971). As such it is important to ensure that these factors are identified and that any possible effects are accounted for during the interpretation of leaf analysis data. Primarily they relate to the age of the crop at sampling, sampling season, the possibility of moisture stress effects and sample collection and handling. As noted earlier variety can also influence leaf nutrient values.

1.3.1. Crop age

As mentioned earlier, the range of third leaf N critical values used in the South African and Guyanian sugar industries recognises that third leaf N declines with crop age. Although this effect is well documented (Evans, 1961, Bishop, 1965; Samuels, 1969), an

innovative investigation by Gosnell and Long (1971) allowed the effects of age and season to be separated. They reported that the third leaf N values declined most markedly in the first few months of growth. In their investigation, third leaf N values declined from a mean value of 2.70% at one month of age, to a mean value of 1.85% at four months of age. From six months of age the rate of decline was substantially reduced (a mean of 1.67%N at this stage to 1.60%N at nine months of age). Although the mean third leaf P, K, Ca and Mg values also declined with age, the rates of reduction were not as marked as those noted with leaf N. Small differences in third leaf P, K and Ca were observed after five months of age.

1.3.2. Season

Season, in the broader context of sugarcane production, usually refers to the period 1May of one year to 30 April of the next year in the southern hemisphere. In terms of leaf analysis it refers to the period in which leaf sampling is applicable. As it is recognised that leaf sampling is only pertinent when the crop is growing actively, the choice of season length should reflect growing conditions in any particular area. In quantifying "active growth", Evans (1965) suggested that conditions should be such that stalk elongation is greater than 20mm/day. Based on this reasoning it is recommended that leaf sampling in the South African sugar industry be undertaken during the period October to April in the northern irrigated areas, but be limited to the period from November to March in the Natal coastal and Midlands regions (Wood, 1989). In the absence of specific guidelines in Australia, Schroeder *et al* (1999) have suggested that active growth will normally occur during the months of November to April in Queensland and December to March in New South Wales. Leaf sampling would therefore be applicable during these periods (seasons), provided enough well distributed rain fell in the month prior to sampling.

1.3.3. Variety

Variety is another factor that appears to affect nutrient uptake and consequently leaf nutrient values. It has been reported from South Africa that the P and K critical values for sugarcane varieties N12 and N14 respectively (Table 1.1) are somewhat lower than those

associated with other N and NCo varieties (Schroeder *et al*, 1993). In an investigation conducted in the Rhodesian (now Zimbabwe) sugar industry, large and significant differences between varieties were observed for third leaf N, P, K, Ca and Mg values in samples collected from a variety trial which included pre-release varieties and the standard NCo 376 variety (Gosnell and Long, 1971). Data from a third ratoon trial in which three commercial varieties (NCo 310, NCo376 and CP 29-116) were replicated 30 times (Gosnell and Long, 1971) showed that significant differences in leaf nutrient values existed between all three varieties (Table 1.3) with adequate nutrition. In particular the mean third leaf N and P values for NCo376 were significantly higher than those of the other two varieties. Similarly it was shown that in Swaziland, the TVD leaf N content of variety NCo376 was generally higher than that of NCo310 and NCo334 for both a summer and winter crop cycle at different sampling ages and times (Table 1.4), and when the varieties were included in trials on six different soils types (du Randt, 1978).

Table 1.3. Foliar analysis values for NCo 310, NCo 376 and CP 29-116 from a variety trial conducted in the former Rhodesia (Gosnell and Long, 1971).

Variety	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
NCo 310	1.92	0.219	1.37	0.307	0.185
NCo 376	1.98	0.227	1.38	0.264	0.180
CP 29-116	1.86	0.199	1.47	0.245	0.235
LSD 5%	0.04	0.007	0.05	0.020	0.012
1%	0.05	0.009	0.06	0.026	0.017
CV %	3.9	5.9	6.3	14.1	12.0

Varietal differences were also reported in the CSR leaf testing system that was operative in the Australian sugar industry during the 1960s and 1970s. In this case it was recognised that considerable differences in critical values (referred to as optimum nutrient indices) existed for various Queensland varieties for N and K expressed as % dry matter, and P as the P:N ratio, using top visible dewlap leaves (Farquhar, 1965). The optimum nutrient index for Q57 was reported to be 95% of that of Pindar for N, 110% for

P and 105% for K. A substantial range in leaf nutrient values has also been reported for varieties grown in Mauritius (Bassereau, 1988).

Table 1.4. Nutrient content of the TVD leaves of three sugarcane varieties (duRandt, 1978).

Nutrient content of TVD leaves (%)										
Crop cycle	Summer									
Age & month of sampling	5 months (May)					9 months (September)				
Nutrients	N	P	K	Mg	Ca	N	P	K	Mg	Ca
Variety										
NCo376	2.00	0.29	1.56	0.21	0.29	1.57	0.18	1.53	0.16	0.24
NCo310	1.99	0.26	1.59	0.21	0.33	1.42	0.18	1.53	0.14	0.27
NCo334	1.88	0.29	1.63	0.21	0.27	1.41	0.19	1.67	0.15	0.24
Crop cycle	Winter									
Age & month of sampling	6 months (December)					9 months (April)				
Nutrients	N	P	K	Mg	Ca	N	P	K	Mg	Ca
Variety										
NCo376	1.61	0.28	1.49	0.18	0.24	2.01	0.28	1.60	0.25	0.32
NCo310	1.59	0.25	1.63	0.18	0.23	1.91	0.27	1.67	0.26	0.36
NCo334	1.50	0.28	1.79	0.17	0.18	1.74	0.27	1.84	0.26	0.33

1.3.4. Moisture stress

Although it is widely accepted that moisture stress affects leaf nutrient values and restricts interpretation of data (Halais, 1962; Evans, 1965; Schroeder *et al.*, 1992), quantitative data relating to the effect of moisture stress is limited. While Samuels (1965) tried to address this issue by simply comparing irrigated and non-irrigated conditions, Gosnell and Long (1971) undertook a more comprehensive study to evaluate the effect of moisture stress on third leaf nutrient values by applying six different irrigation treatments to a third ratoon crop of variety NCo376. As expected the N content of the third leaf samples declined with increasing moisture stress expressed as days between irrigation and sampling (Table 1.5). It was also reported that third leaf P values slowly decreased with increasing moisture stress. While third leaf K values were affected by severe

moisture stress, there was little difference between the fully irrigated and moderately water stressed treatments (Gosnell and Long, 1971). In addition, while the leaf Ca content increased with severe moisture stress, Mg values did not show any definite trend.

Table 1.5. Third leaf N content as affected by moisture deficit at sampling (Gosnell and Long, 1971).

Day between irrigation and sampling	Class A Pan moisture deficit (mm)	Third leaf N (%)
5	24	1.96
5	24	1.95
7	35	1.92
7	35	1.91
8	40	1.92
13	66	1.84

In order to avoid any moisture stress effect, sampling guidelines emphasise the need to collect samples only where moisture stress is non-limiting (Halais, 1962) ie. when the crop has received enough well distributed rainfall and/or irrigation to preclude any moisture stress.

1.3.5. Prerequisites for leaf sampling

In view of the effect of the different factors that may influence leaf nutrient values, a number of requirements or prerequisites exist to enable meaningful interpretation of leaf analysis data. These are as follows:

- The date of sampling must fall within the prescribed sampling period for the area/region to ensure that leaf samples are collected from actively growing cane.
- The cane age at the time of sampling must fall within prescribed limits to ensure the applicability of the established critical values (Tables 1.1 and 1.2).
- Six weeks must have lapsed since any fertiliser application to ensure enough time for uptake of available nutrients.

- Enough well distributed rain must have fallen and/or sufficient irrigation needs to have been applied to ensure no moisture stress effects prior to sampling and to ensure uptake of applied nutrients.
- Vigorous plant growth must have occurred in the month prior to sampling.
- Sugarcane being leaf sampled should not be affected by any other factor relating particularly to disease, insect damage and/or waterlogging.

1.3.6. Sample collection

Sampling procedures for collecting leaf material from sugarcane crops and a list of details that need to be recorded at that time of sampling have been developed around the world. Fortunately, these have converged into more or less standard guidelines that are followed in most of the world sugar industries. Apart from minor modifications that may be applicable in some countries, the following is recognised as the recommended sampling procedure:

- Select leaves from stalks of average height.
- Sample the third leaf from the top of the stalk. The first is the one that is more than half unrolled.
- Collect about 40 such leaves from the field (block) of sugarcane, preferably using a diagonal sampling pattern.
- Fold the leaves in half (tip to base) and cut a 100 to 150 mm length from the folded leaf (giving a total of 200 to 300 mm length of lamina).
- Strip out and discard the midrib from each 200 to 300mm length of lamina.
- Tie the ± 80 lamina sides (from the forty leaves) into a bundle and place in a clean paper bag. Keep this composite sample in a cool environment (polystyrene cooler) until the sample can be dried in an oven, or a well-ventilated area.
- Once the sample is dry, send it to a reputable laboratory for analysis.
- Ensure that each sample is properly identified and supply details of variety, crop (plant or ratoon number), sampling date and age of cane at harvest, and details of fertiliser applied (type and rate).
- Avoid any contamination whether it be at the time of sampling, drying or storage.

1.4. Leaf analysis: Advances in interpretation and uses for advisory and nutrient trend purposes

Over the years, a number of advances have been made that have improved leaf analysis from being purely diagnostic to a more fully encompassing 'tool' for advisory and nutrient trend purposes. These improvements have not only been related to the scope of sophistication but also to modifying third leaf critical values where necessary.

1.4.1. Modified critical (threshold) values

As a result of a continuing research, development and/or extension effort, specific circumstances are sometimes identified where established critical values appear to be no longer fully appropriate for use across regions, varieties, soil type, etc. In such cases growing evidence may suggest that modified critical values should be introduced. For instance, a large number of leaf analysis data from winter cycle sugarcane grown on the base saturated clay soils of the irrigated areas of the South African sugar belt (Mpumalanga and Pongola) indicated that K uptake was depressed during the spring months of each season (Donaldson *et al*, 1990). Following extensive investigation, a seasonal correction factor for leaf K critical values was introduced within the SASEX fertiliser advisory service to account for Ca and Mg antagonism, the effect of reduced K uptake during periods of relatively low temperature, and varietal differences under such conditions (Donaldson *et al*, 1990). As a result, the current third leaf K critical value for all varieties (except N14) grown as winter-cut irrigated cane is 0.85% if samples are collected during mid October to November (Table 1.6). This value increases to 0.95% K for December and January sampling, and to the established value of 1.05% for samples collected in February to April. Variety N14 has a third leaf K critical value 0.15 percentage units lower than that applicable to the other varieties (Table 1.6).

Previous modifications, particularly in relation to third leaf N values, are shown in Tables 1.1 and 1.2. Reported modification to third leaf critical values due to varietal differences was discussed earlier in section 1.3.3.

Table 1.6. Modified third leaf K critical values for winter cut irrigated cane (Schroeder *et al*, 1992).

Age of cane at sampling (months)	Sampling period	Third leaf K critical value (%)	
		All N and NCo varieties except N14	N14
3 – 5	mid Oct – Nov	0.85	0.70
	Dec – Jan	0.85	0.80
	Feb - Apr	1.05	0.90

1.4.2. Diagnosis and Recommendation Integrated System (DRIS) indices

The DRIS system that was developed by Beaufils (1973) was an attempt to add the concept of balanced nutrition to the interpretation of leaf (and soil) analysis for diagnostic and advisory purposes. The nature of the system questioned the use of single-valued critical values as the optimum concentration of a particular nutrient was considered to be dependent on its interaction with and concentration of other nutrients (Bassereau, 1988). With the system, the so-called DRIS indices are calculated from nutrient ratios. Various evaluations of DRIS for use with sugarcane have indicated that although these indices are probably more efficient in detecting nutrient imbalances and deficiencies than conventional critical values in young cane (Meyer, 1981; Ng Kee Kwong and Deville, 1983), the system has never been widely used in sugarcane production. This lack of acceptance has most often been ascribed to the diminished sensitivity of the indices with crop age (Meyer, 1981), a fact that particularly limits the use of the system in the South African sugar industry where leaf samples are generally collected from cane that is four to seven months of age in the rain-fed regions. However, DRIS is considered applicable in the warmer northern irrigated areas of the industry where generally younger cane (three to five months of age) is leaf sampled. Here N imbalances in particular would be detected sooner with DRIS than with the conventional critical value approach (Meyer and Wood, 1982). Another disadvantage of the DRIS is an apparent absence of information relating to rates of supplementary nutrient applications when using the system.

1.4.3. Crop logging

In Hawaii much effort has been devoted to the development of the sugarcane crop log system (Clements, 1959). It was established in the 1940s with the aim of providing a means of detecting and correcting any nutrient (and/or water) deficiency with minimum delay (Whalley and Clarkson, 1950). With this system, samples (leaves 3 – 6 for N analysis and their sheaths for fresh weight, moisture, total sugars, P, K, Ca and Mg) are collected every 35 days. The resulting nutrient indices for N, Ca and Mg (as a percentage of dry matter), and P and K (as percentages of sugar-free dry matter) are recorded and charted throughout the life of the crop (Bassereau, 1988). Based on the comparison of these indices with desirable index values, fertiliser applications would be recommended at various stages of the crop to alleviate any deficiencies that were identified as the crop progressed. Despite the advantages of the system, the very intense sampling program associated with the system makes it difficult to implement in practice.

1.4.4. CSR leaf testing service

During the 1960s and 1970s, the Australian commercial sugar company CSR (Ltd), developed a leaf testing service to provided fertiliser recommendations for growers supplying cane to their mills (Farquhar, 1965). Advice was based on optimum leaf nutrient indices and a number of nutrient action levels (range of values above and below the optimum nutrient indices). These were updated annually based on the results of a number of NPK factorial trials in each mill area. Analysis data of leaf samples from individual blocks were compared to the appropriate action levels (based on variety) to determine appropriate adjustments to previous fertiliser application rates. For instance, the 1973 nitrogen fertiliser advice for the Herbert River district (Table 1.7) was based on the action levels that had been compiled from the results of the 1972 trials.

When using this system, a third leaf analysis value of 2.15% for a particular block of cane, for example, would have resulted in no change to the N fertiliser programme (as used for the previous crop), if the variety was Triton. However for variety Q68 and Pindar, the recommendation would have been respectively 52 kg/ha **less** and 52 kg/ha **more** than the previous application rates.

Due to an apparent lack of support by the grower community and as a result of resources being required elsewhere, this service was withdrawn during the mid 1970s.

Table 1.7. CSR nitrogen action levels: Herbert River district - 1972 (CSR (Ltd)) unpublished data – Report on technical field work 1972 – 1973).

Sugarcane variety			Recommendation
Pindar	Triton	Q68	
	Leaf N (%)		
> 2.49	> 2.30	> 2.29	Previous N rate – 103 kg/ha
2.35 – 2.49	2.16 – 2.30	2.15 – 2.29	Previous N rate – 52 kg/ha
2.20 – 2.34	2.01 – 2.15	2.00 – 2.14	Previous N rate + 0
2.05 – 2.19	1.86 – 2.00	1.85 – 1.99	Previous N rate + 52 kg/ha
1.90 – 2.04	1.71 – 1.85	1.70 – 1.84	Previous N rate +103 kg/ha
< 1.90	< 1.85	< 1.70	Investigate

1.4.5. SASEX whole cycle fertiliser advice

The South African Sugar Association Experiment Station (SASEX) has conducted a fertiliser advisory service (FAS) for cane growers since 1954. Based on the analysis of soil samples collected prior to planting / replanting, it provides growers with 'whole cycle' fertiliser advice for a cycle of plant crop and four succeeding ratoons. Analysis results of leaf samples taken during the crop cycle are then used to check the adequacy of the original recommendations (Schroeder *et al*, 1993) according to the locally established critical values (Tables 1.1 and 1.2). In this way leaf analysis is used as a basis confirming or correcting the fertiliser programme for the subsequent crop. However, guidelines for additional fertiliser applications to the current crop are also available for N, P and K (Table 1.8). The proviso exists that the cane being sampled should be young enough (3-5 months of age) to enable effective crop utilisation of any supplementary nutrient dressings. Equally important, leaf analysis has been used as a diagnostic tool for determining possible nutritional causes of poor crop growth and/or imbalances in sugarcane crops (du Toit, 1959; Schroeder *et al*, 1993).

Table 1.8. Recommendations for additional N, P and K application based on leaf analysis in the South African sugar industry (Anon., 1996).

	Leaf nutrient value (%)			
	Nitrogen			
Leaf N (%)	<CV* – 0.4	CV – 0.4 to CV – 0.2	CV – 0.2 To CV	>CV
Additional N required (kg/ha)	100	75	50	0
	Phosphorus			
Leaf P (%)	<CV – 0.03	CV – 0.03 to CV – 0.02	CV – 0.01 To CV	>CV
Additional P required (kg/ha)				
Weakly P sorbing soils	30	20	20	0
Strongly P sorbing soils	80	50	30	0
	Potassium			
Leaf K (%)	<CV – 0.2	CV – 0.2 to CV – 0.1	CV – 0.1 To CV	>CV
Additional K required (kg/ha)	150	100	50	0

* Critical value

1.4.6. Nutrient surveys

Nutrient surveys based on leaf analysis offer a useful way of determining nutrient trends at various levels in an industry. Just as a grower can usefully employ leaf analysis data to determine nutrient changes on his farm, so too the composite use of leaf sample analysis results can lead to the identification of nutrient trends at regional or whole industry level. Knowledge of increasing, decreasing or relatively constant supply of crop nutrients is not only important from a production point of view but provides a good basis for research, development and extension activities in any agricultural industry. With the increased use of inorganic fertiliser from the early 1950's, much emphasis in terms of the overall research effort and on-farm management was directed at the use and maintenance of the macro and secondary nutrients. However, as it was recognised that the micro nutrients were considered equally essential for healthy plant growth, a nutrient survey based on leaf analysis was conducted in the South African sugar industry in the early 1970s

(Meyer *et al*, 1971). This survey had the particular purpose of assessing the micro-nutrient status of the sugarcane crop on an industry-wide basis and locating potentially nutrient deficient areas. The leaf analysis section of this survey indicated that no widespread trace element deficiencies occurred (Meyer *et al*, 1971), though zinc and manganese were respectively shown to be deficient in 11,7% and 2.2%of all the samples included in the survey (Table 1.9).

Table 1.9. Average nutrient content of leaf samples for various physiographic regions (Meyer *et al*, 1971).

Physiographic regions	No. of samples	B	Cu	Zn	Mn	Al	Fe
				(ppm)			
Coastal lowlands	228	4.1	6.9	18.3	48	83	146
Midlands mistbelt	135	4.0	7.2	14.9	74	133	163
Sub-humid midlands	36	2.0	6.9	17.1	67	60	103
Lowveld:							
Pongola	13	2.6	7.5	15.6	42	40	91
Swaziland	21	4.9	8.0	18.8	25	165	196
Eastern Transvaal	39	4.4	7.6	17.4	38	112	182
Natal	15	3.5	6.1	23.9	35	132	173
Total	487						
Range	Lowest	1.6	4.2	10.0	11	21	49
	Highest	10.0	12.2	55.3	270	800	915
Threshold value		1	3	14	15	-	50
No: deficient samples		Nil	Nil	57	11	-	1
% of total deficient		Nil	Nil	11.7	2.2	-	<1

Subsequent to this survey, but prior to the computerisation of the SASEX fertiliser advisory service in 1980, little use was made of analytical data for determining trends in soil fertility and plant nutrition (Meyer *et al*, 1998). However with computerisation came the facility of enabling regular interrogation of the data base that consisted of all analysis results pertaining to growers' leaf (and soil) samples (Meyer *et al*, 1989). By 1998 the leaf analysis data base, referred to as the Nutrient Information Retrieval System (NIRS) had grown to include more than 70 000 growers leaf sample results (Meyer *et al*, 1998). A 1989 report on nutrient trends in the industry indicated that a relatively high proportion of leaf samples (28%) were deficient in K (Table 1.10) and

that 12 to 13% of the samples showed low N and P values (Meyer *et al*, 1989). Incidences of Ca, Mg and Zn deficiencies appeared to be low across regions.

Table 1.10. Mean third leaf nutrient values for the various bioclimatic regions in the South African sugar industry (Meyer *et al*, 1989).

Natural region	Nutrient values					
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (mg kg^{-1})
Coastal lowlands (Berea system)	1.84	0.21	1.21	0.27	0.22	18
Coastal lowlands (Umzinto system)	1.87	0.22	1.20	0.26	0.26	18
Coastal hinterland	1.95	0.22	1.42	0.29	0.25	17
Midlands mistbelt	1.94	0.20	1.29	0.26	0.24	18
Lowveld	2.03	0.25	1.32	0.34	0.27	19
Critical value	1.70	0.17	1.05	0.15	0.10	13
% sample deficient	13	12	28	4	1	8

Although it has more recently been noted that the incidences of N and P deficiency have increased and that there is evidence of luxury uptake of K (Meyer *et al*, 1998), there is no justification for suspecting large scale micro-nutrient deficiencies in the South African industry (Meyer *et al*, 1999).

1.5. Conclusions

The following conclusions were drawn:

- Although there is widespread and on-going development and use of leaf analysis for diagnostic, advisory and nutrient trend purposes in various sugarcane industries worldwide, some countries have developed more sophisticated systems than others.
- Fairly robust critical values covering macro, secondary and micro nutrients exist for use across industries, regions and varieties.
- There has been substantial modification of the third leaf critical values, based on sampling period, age of cane at sampling and variety.

- Although most of the factors affecting leaf analysis have been fairly rigorously investigated, there appears to be little quantitative information available regarding the effect of moisture stress on the nutrient content of sugarcane.
- The current strategy in the use of leaf analysis is to recommend that sampling is carried out when conditions are favourable for optimum plant growth and hence precluding any moisture stress effects.
- Although this avoidance technique is the most suitable option in the absence of definitive data, it does not provide a solution to interpreting leaf analysis data affected by moisture stress or understanding the nutrient content of sugarcane under such conditions.
- The lack of supporting data for moisture stressed conditions fundamentally restricts the use of leaf analysis applications such as trend analyses and nutrient surveys as they are currently dependent on samples collected from unstressed cane.
- To ensure more widespread use leaf sampling and meaningful interpretation of leaf analysis data, it is considered essential that there is a greater understanding of the nutrient content of sugarcane under moisture stress conditions, particularly in relation to the third or top visible dewlap leaves.
- The development of a moisture stress index that could be used in association with leaf analysis would not only allow greater confidence in interpreting leaf data but also possibly broaden the appropriate sampling period and lift constraints on sampling prerequisites.

Chapter 2.

Leaf nutrient values as affected by moisture stress – evidence from the South African sugar industry.

2.1. Introduction

Due to the requirements for meaningful interpretation of leaf analysis data (as indicated in Chapter 1), sugarcane growers are advised to leaf sample only when conditions are favourable and the required growth rate of the cane is assured ie. when enough well distributed rainfall has occurred or sufficient irrigation has been applied to preclude any moisture stress effects prior to sampling. However, it has been reported that many of the large commercial cane growing enterprises including miller-cum-planter operations and larger estates have found it difficult to adhere to these prerequisites as their yearly programmes are planned well in advance. Dates for leaf sampling are set within fairly rigid timetables. In view of this, large numbers of leaf samples were received by the SASEX fertiliser advisory service laboratory during the early 1990s, despite the below average and seasonally variable rainfall that occurred in the South African sugar industry at that time.

From anecdotal evidence it appeared that moisture stress may have been an important factor influencing leaf nutrient values during the drought conditions that have fairly regularly affected the South African sugar industry. As part of the routine quality control process and assessment of leaf analysis results leaving the laboratory, nutrient values associated with growers' samples were (and continue to be) screened in order to identify any moisture stress effects.

2.2. Materials and methods

Relevant analysis results of leaf samples submitted by growers to the FAS laboratory in conjunction with data from the industry-wide meteorological stations were used to evaluate whether low rainfall had any effect on leaf nutrient values.

2.2.1. Examples to illustrate the possible effect of moisture stress on leaf analysis data

Examples were used to illustrate this effect by utilising data that were

- Associated with sugarcane fields that had been sampled during possible moisture stress conditions and again once the moisture stress conditions had dissipated (Example 1).
- Related to adjacent fields where the moisture stress effect appeared to be different (Example 2).
- Available at a regional level via the NIRS (Example 3).

In order to evaluate whether these somewhat qualitatively determined trends of low leaf nutrient levels were indeed related to drought conditions, two "whole farm" investigations were conducted using leaf analysis and rainfall data. In these two case studies, the growers had regularly leaf sampled over a number of years covering both 'normal' and drought affected seasons.

2.2.2. Case study 1 (To determine the effect of moisture stress on leaf nutrient values on a whole-farm basis):

The farm was situated in Zululand and had a predominance of Kroonstad form (orthic A horizon overlying an E horizon over a gley-cutanic B horizon) and Westleigh form (orthic A horizon overlying soft plinthite) soils (Macvicar *et al*, 1977). Analysis data associated with thirty representative leaf samples collected by the grower from various fields across the farm over three seasons (1988/89, 1989/90 and 1990/91) were included in the study. The ten samples collected each year were considered as being sub-samples of the whole farm (which represented

the whole sampling area but divided according to soil form). The data was assessed by analysis of variance.

2.2.3. Case study 2 (To determine whether the effect of moisture stress on leaf nutrient values (on a whole farm basis) was related to broad soil type and/or variety):

In this case study, the farm was in the Natal Midlands and consisted of Inanda/Nomanci form (humic A horizon over either red apedal B or lithocutanic B horizons) and Cartref or Grenrosa form (orthic A horizon overlying an E horizon over a lithocutanic B horizon or orthic A horizon over a lithocutanic B horizon respectively) soils (Macvicar *et al*, 1977). For simplicity the different soil types were grouped together according to their parent materials to form two broad soil categories. The Inanda/Nomanci form soils were collectively assigned as Table Mountain Sandstone - Mistbelt (TMS-M) soils and the Cartref/Glenrose form soils as Table Mountain Sandstone - Ordinary (TMS-O) soils (Beater, 1957; Anon., 1984). Leaf analysis data from 16 fields of ratoon cane (variety N12) that had been repeatedly sampled in the 1988/89 (or 1987/88), 1989/90 and 1990/91 seasons were included in the case study and assessed by analysis of variance. The leaf analysis data pertaining to 1987/88 and 1988/89 were grouped together as both seasons were generally considered to be suitable for leaf sampling with little chance of moisture stress affecting leaf nutrient values.

In both case studies the mean nutrient applications rates were calculated from the information supplied by growers on the leaf sample labels. Soil form for each field was determined from FAS records.

2.3. Results and discussion

A total of 7789 growers' leaf samples were received by the FAS laboratory from October 1989 to June 1990 (Figure 2.1). This number compared favourably with the number of samples submitted annually over the previous three years, despite the

unfavourably dry conditions that characterised much of the 1989/1990 growing season (Table 2.1). Although the total rainfall during the period November 1989 to April 1990 exceeded the long-term mean, precipitation was not well distributed and was lower than the long-term means for December, January and February. These unseasonally dry months did not contribute to favourable leaf sampling conditions in much of the industry and hence, in retrospect, many of the samples submitted for analysis were affected by moisture stress and showed low nutrient values.

Although the growing season of the following year (1990 to 1991) started with below average rainfall in November, good well-distributed rain fell in the period December to March, resulting in a good season for leaf sampling. As severe drought occurred during much of the 1991/92 season, growers were discouraged from leaf sampling especially after January 1992, except where the irrigation water was sufficient to ensure unstressed cane at the time of sampling. Consequently, the numbers of leaf samples submitted to the FAS laboratory decreased from 8269 in 1990/91 to 4234 in 1991/92 (Figure 2.1).

Figure 2.1. Number of growers' leaf samples received by the FAS during the 1986/87 to 1990/91 growing seasons

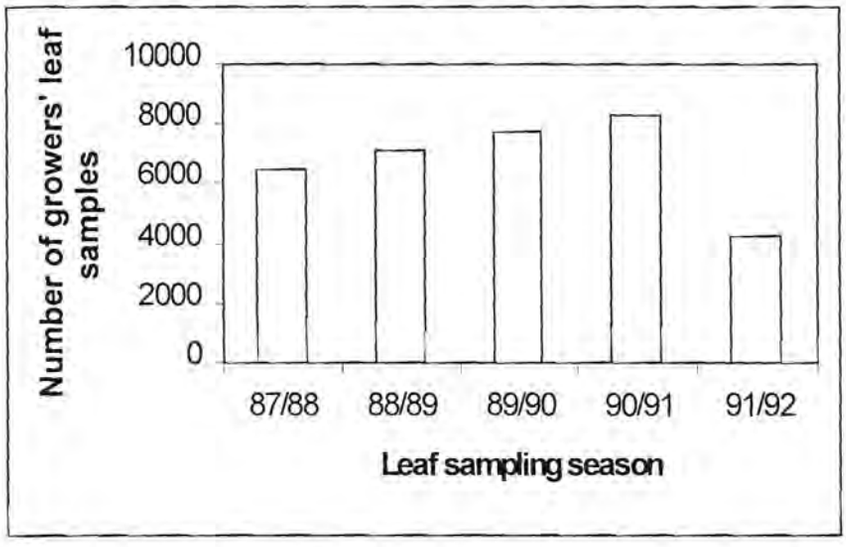


Table 2.1. Mean rainfall figures for the South African sugar industry during the growing seasons 1986/97 to 1990/91.

	Industry rainfall (mm)						
	Nov	Dec	Jan	Feb	Mar	Apr	Total (Nov – Apr)
1986/87	100	160	152	70	170	41	693
1987/88	148	91	73	303	248	28	891
1988/89	107	175	58	259	36	85	720
1989/90	341	97	67	91	210	65	871
1990/91	54	147	140	156	177	14	688
1991/92	107	77	82	41	52	33	392
Long-term mean	111	114	119	124	125	67	660

In general, it was noted that unusually low nutrient values were associated with a large proportion of the leaf samples collected from the rain-fed regions of the South African sugar industry (Zululand, the Natal Midlands, and the North and South Coast regions of KwaZulu-Natal) during the 1989/90 season. This trend occurred despite apparent adequate fertiliser application in most instances, and general adherence to the recommended sampling guidelines in terms of sampling age and date. Three specific examples are used here for illustrative purposes:

2.3.1. Example 1 (sampling during possible moisture stress conditions and again once the moisture stress conditions had dissipated):

The analysis of leaf samples from adequately fertilised cane on an estate on the Zululand Coast, showed low to very low N, P and K values (Table 2.2). The recorded rainfall for the three-month period that preceded sampling was well below the long-term mean for the area (as recorded at the Amatikulu weather station) although heavy rains had fallen during November 1989 (Table 2.3)

Leaf samples taken from the same fields after the 'good' rainfall events late in the season (Table 2.3), indicated substantial improvement in the nutrient status (above the relevant critical values shown in Table 1.1) despite the cane being two months older than at the previous sampling (Table 2.2). The improvement in the leaf N, P and K values confirmed that the low values were not associated with nutrient deficiencies *per se*, but rather with moisture stress effects associated with the unusually low rainfall. The possibility of large nutrient losses following the November rains was also excluded.

Table 2.2. Third leaf nutrient values of samples collected on a Zululand farm in February and May 1990.

Sampling Date	Field No.	Variety	Crop	Age (mnths)	Third leaf nutrient values (%)		
					N	P	K
20 Feb 90	48	NCo367	5	5	** 1.48	***0.12	* 0.90
	49	Mixed	5	5	** 1.44	***0.14	**0.83
	50	NCo376	7	5	***1.30	***0.12	**0.83
	66	Mixed	2	4	***1.32	***0.14	1.31
24 May 90	48	NCo367	5	5	1.71	0.18	1.46
	49	Mixed	5	5	1.87	0.19	1.32
	50	NCo376	7	5	1.61	0.19	1.43
	66	Mixed	2	4	1.76	0.20	1.520

*Marginal, ** Low, *** Very low (according to the critical values shown in Table 1.1)

Table 2.3. Recorded rainfall for the period November 1989 – March 1990 (Amatikulu weather station).

Day	Measured Rainfall (mm)				
	Nov 89	Dec 89	Jan 90	Feb 90	Mar 90
1-2	6.0	14.4			12.2
3-4	15.8				20.4
5-6	3.6	4.0	1.6	23.4	
7-8	12.6	26.2	2.4		
9-10	11.2	0.2	8.2	3.2	0.8
11-12	3.6		3.4	1.2	11.8
13-14		7.2		11.0	1.2
15-16	17.6	2.0		18.0	6.4
17-18	0.8				9.8
19-20		2.0	3.4	0.8	
21-22	2.6			0.4	1.0
23-24	0.8	2.2	26.8		81.4
25-26	17.8	2.6	4.0	4.2	5.2
27-28	18.6		7.6		7.2
29-30	333.0	9.2			
31					4.0
Total	444.0	70.0	57.4	62.2	161.4
Long-term mean	123.5	92.0	134.3	138.6	124.8

2.3.2. Example 2 (sampling adjacent field where moisture stress affects were different):

Leaf samples taken from 20 fields (identified here as 1 – 20) on a commercial enterprise in the Natal Midlands during February 1990 (Table 2.4) indicated low leaf N and some marginal P values associated with sugarcane grown on Cartref form soils (shallow coarse textured, low organic matter soils derived from Table Mountain Sandstone) despite adequate fertiliser application. In contrast, samples from adjacent fields on Inanda form soils (deep humic sandy loams) showed satisfactory N, P and K values in similar circumstances. While N values associated with the two soil types were significantly different at the 5% level, the P and K values were not significantly different. The fact that the sugarcane on the Cartref soils reportedly 'greened-up' and recovered dramatically after the well

distributed rainfall events that occurred during March 1990 suggested that the low N values were related to the moisture stress effect caused by the below average rainfall during December to February 1990 (Table 2.5). It appeared that cane grown on the deep humic soils was better able to withstand the effects of low rainfall than the Cartref soils, presumably due to better water-holding capacities.

Table 2.4. Third leaf nutrient values of samples collected from a commercial enterprise in the Natal Midlands during February1990.

Soil type	Field No.	Variety	Crop	Age (mnths)	Third leaf nutrient values (%)		
					N	P	K
Cartref	1	N12	4	4	* 1.50	0.19	1.24
	2	N12	4	4	** 1.44	0.19	1.16
	3	N12	3	8	***1.35	* 0.16	1.21
	4	N12	4	8	* 1.56	0.21	1.49
	5	N12	2	8	* 1.54	0.17	1.24
	6	N12	2	8	* 1.61	* 0.16	1.22
	7	N13	3	4	* 1.52	0.19	1.11
	8	N13	3	4	** 1.47	0.20	1.03
	9	N12	2	8	* 1.55	0.17	1.27
	10	N12	2	8	* 1.54	0.18	1.33
	Mean				1.508	0.182	1.231
	SE				0.073	0.017	0.124
Inanda	11	NCo293	3	7	2.12	0.19	1.00
	12	NCo293	3	5	2.09	0.21	1.31
	13	NCo293	3	7	2.30	0.22	1.13
	14	N12	4	5	1.92	0.19	1.22
	15	NCo293	4	6	1.90	0.20	1.14
	16	N12	1	3	2.02	0.23	1.30
	17	N12	2	7	1.93	0.21	1.14
	18	N12	1	4	1.88	0.21	1.10
	19	N12	2	7	1.85	0.18	1.13
	20	N12	3	6	1.74	0.17	1.08
	Mean				1.975	0.201	1.155
	SE				0.161	0.019	0.096

* Marginal , ** Low, *** Very low (according to the critical values shown in Table 1.1)

Table 2.5. Recorded daily rainfall for the period November 1989 – March 1990 (Beaumont weather station).

Day	Recorded Rainfall (mm)			
	Dec 89	Jan 90	Feb 90	Mar 90
1-2	2.5			
3-4	1.0			8.3
5-6	4.0	6.5	9.0	30.0
7-8	5.5		2.5	1.0
9-10		4.0	1.5	1.5
11-12			1.5	13.5
13-14	21.0	2.5	13.0	
15-16	1.5		13.5	11.0
17-18				13.0
19-20	4.0	32.0	1.5	7.5
21-22	1.0	1.5	2.5	
23-24	2.5	12.0		50.0
25-26	0.8	1.0	7.3	35.0
27-28	1.8	7.0		1.0
29-30	4.5			
31	3.5			2.0
Total	53.6	66.5	51.3	173.8
Long-term mean	117	95	167	106

2.3.3. Example 3 (using data at the regional level):

Based on the analysis of about 150 samples per annum, the NIRS data pertaining to the lower Natal South Coast region indicated that the mean third leaf N value for the 1989/90 season was substantially lower than the mean third leaf N values for the periods covering 1983 – 1985 and 1986 – 1988 (Table 2.6). In 1989/90 the percentage of samples that showed apparent deficient N values increased to 48% from the 21% and 20% indicated for the previous two periods. Only 29% indicated adequate leaf N values in 1989/90 compared to 52% during 1983 to 1988. As with the other rain-fed areas of the industry, these apparent increases in N deficiency were attributed to the low monthly rainfall that occurred during the

first three months of 1990 (Table 2.7) rather than an N deficiency *per se*. In the case of the 1989/90 season the three critical months for good sampling conditions (December, January and February) were all characterised by extremely low rainfall, giving a total of only 212mm for the summer months (Table 2.7). The substantial improvement in the mean leaf N value for the region (to 1.89%N) in the 1990/91 season (Table 2.6) with the improved rainfall distribution over the summer months (Table 2.7), confirmed that the mean leaf N value for the previous season was moisture-stress induced rather than the result of an actual nutrient deficiency. In 1990/91 the percentage of samples classified as adequate had improved to 62% without widespread changes in fertiliser management.

Table 2.6. Mean third leaf N value and percentage of samples per category (deficient, marginal, adequate and high) for the lower Natal South Coast (1983 – 1991).

Sampling Period	Mean third leaf N value (%)	Percentage of samples per category (%)			
		Deficient	Marginal	Adequate	High
Category limits		<1.6	1.6 – 1.8	1.8 – 2.7	>2.7
1983 – 1985	1.86	21	22	52	2
1986 – 1988	1.85	20	25	52	0
1989 – 1990	1.69	48	23	29	0
1990 - 1991	1.89	15	23	62	0

Table 2.7. Recorded rainfall at Umzimkulu (lower Natal South Coast) for the period December to February during the seasons 1983/84 to 1990/91.

	Recorded rainfall (mm)							
	83/84	84/85	85/86	86/87	87/88	88/89	89/90	90/91
Dec	100.5	31.4	90.3	120.0	80.9	168.7	89.1	98.0
Jan	321.9	86.3	144.8	123.8	96.5	40.0	50.9	107.5
Feb	145.3	332.1	31.0	37.1	290.8	307.3	72.0	78.5
Total (Dec – Feb)	567.7	449.8	266.1	370.1	468.2	516.0	212.0	284.0

2.3.4. Case studies 1 and 2:

Meteorological data pertinent to Zululand and the KwaZulu-Natal Midlands, as measured at the Amatikulu and Beaumont weather stations respectively, indicated that the recorded rainfall during the 1989/90 leaf sampling season was substantially lower than in 'normal' years. In particular 192mm of rainfall was measured at Amatikulu during December 1989 and January and February 1990 (Table 2.8). This was about 170mm less than the long-term mean rainfall (365mm) for the area over this period. Comparably, 375mm and 367mm of rainfall were recorded respectively during the same period in 1988/89 and 1990/91. Similarly, below average rainfall was experienced in the Natal Midlands during the summer of 1989/90, with the 171mm of recorded rainfall at Beaumont (December to February) being about 200mm less than the long-term mean rainfall (368mm) for the area. During 1987/88, 1988/89 and 1990/91, 390mm, 392mm and 382mm were recorded respectively for the same three month period in each season.

Table 2.8. Recorded rainfall for the months of December, January and February of the 1989/88 to 1990/91 seasons at the Beaumont and Amatikulu weather stations.

	Recorded rainfall (mm)							
	87/88		88/89		89/90		90/91	
	Beaumont	Amatikulu	Beaumont	Amatikulu	Beaumont	Amatikulu	Beaumont	Amatikulu
Dec	106.7	105.6	143.9	147.6	53.6	70.0	121.9	140.6
Jan	54.5	62.6	68.1	31.4	66.5	59.4	123.6	86.8
Feb	228.7	330.6	179.7	196.2	51.3	62.2	135.6	139.2
Total	389.9	498.8	391.7	375.2	171.4	191.6	381.1	366.6

In both case studies the year (season) of sampling had a significant effect on leaf nutrient values. Data from the Zululand farm showed that the mean leaf N value associated with the 1989/90 samplings was significantly lower than the leaf N values of the samples collected during both the 1988/89 and 1990/91 seasons

(Figures 2.2(a)) even though fertiliser application rates were essentially unchanged during this period (Table 2.9). Similarly, the leaf N values associated with the 1989/90 samples from the Midlands farm were significantly lower than the N values of samples taken from the same fields during 1987 to 1988 and the 1990/91 sampling period. However, in this case the effect was dependent on soil type (Figure 2.3(a)). With TMS-O soils, leaf N values of samples collected in 1989/90 were significantly lower than those of the earlier (1987-1988) and later (1990/91) sampling periods. In contrast, no such depression in leaf N values was noted in samples associated with the TMS-M soils over this period.

In relation to leaf P, a significant difference was observed between the mean leaf P values associated with samples collected on the Zululand estate in 1988/89 and 1989/90 (Figure 2.2(b)). However, unlike expected, the mean value did not increase above the critical value of 0.19% the following year once the drought conditions had dissipated. This phenomenon can, at least in part, be explained by the fact that the samples were more biased towards variety N12 which has an accepted third leaf P critical value of 0.16% (Schroeder *et al*, 1993). The leaf P data from the Midlands estate indicated an interactive effect between year of sampling and soil type (Figure 2.3(b)). No significant difference was apparent between the mean leaf P values associated with the samples collected from the cane grown on the TMS-M soils for the years under consideration. However, in relation to the TMS-O soils, the mean leaf P value of the 1989/90 season samples was significantly lower than that of the 1988/89 season (Figure 2.3 (b)). Although the mean leaf P value for the 1990/91 season was not significantly different from that of the previous year, it had improved substantially to a value (0.176%) which was not significantly different from that of the 1988/89 season (0.186%).

The third leaf K values associated with the Zululand farm appeared to be unaffected by the sampling season and hence drought conditions in 1989/90 (Figure 2.2(c)). This apparent lack of sensitivity of leaf K values to moisture

stress conditions was not in conflict with anecdotal evidence that indicated that crops behaved differently in relation to K status of third leaf samples in the first year of drought conditions. In some circumstances, third leaf K values appeared to remain more of less stable (or decline slightly), whereas in other cases the K values increased considerably. An explanation may be that in moisture stress conditions, a plant will take up increased amounts of potassium in order to improve the osmotic potential to favour water consumption at the root/soil interface (Marschner, 1993). The highly significant decline in the mean leaf K value in 1990/91 compared to the previous two seasons was attributed to a change in K fertiliser applications on the farm. Although potassium fertiliser was applied at a fairly constant rate over the three year period on the Kroonstad form soils (150-160 kg K ha^{-1}), the K applied on the Westleigh form soils decreased to about 125 kg K ha^{-1} in the 1990/91 season (Figure 2.4). In relation to the Midlands farm, no difference in mean leaf K values was observed in samples collected from cane grown on TMS-O soils. In contrast the samples collected from sugarcane grown on TMS-M soils showed a significantly increased third leaf K value in 1989/90 compared to mean leaf K values of the other two seasons.

Table 2.9. Mean nutrient application rates associated with the fields of sugarcane which were leaf sampled.

		Mean nutrient application rate (kg N, P or K ha^{-1})		
Estate	Nutrient	1988/89	1989/90	1990/91
Zululand	N	122	124	121
	P	0	0	3
	K	156	156	138
Midlands	N	127	134	139
	P	36	38	37
	K	153	142	144

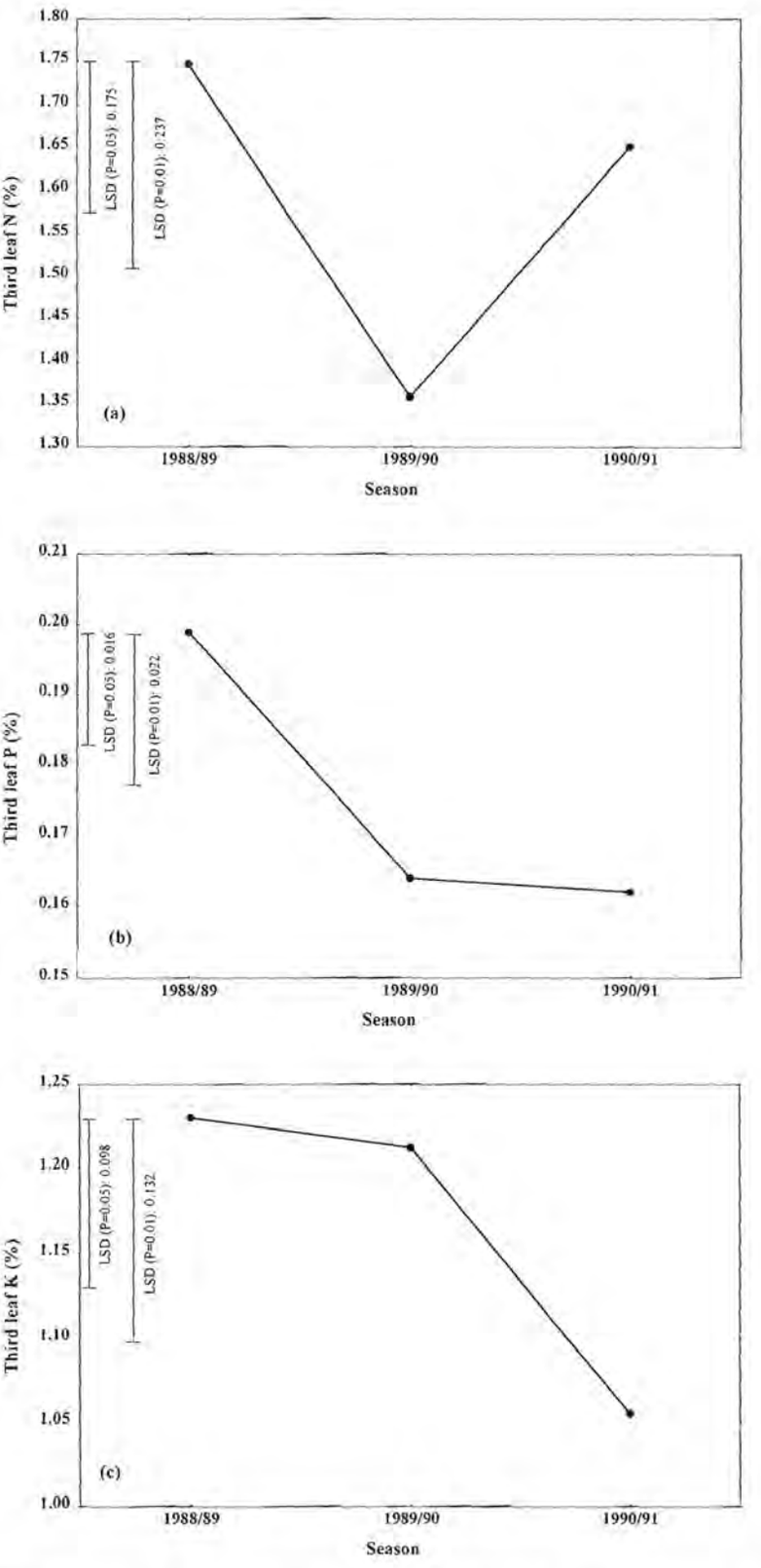


Figure 2.2. Leaf nutrient values associated with the samples collected from the Zululand estate during the 1988/89, 1989/90 and 1990/91 seasons.

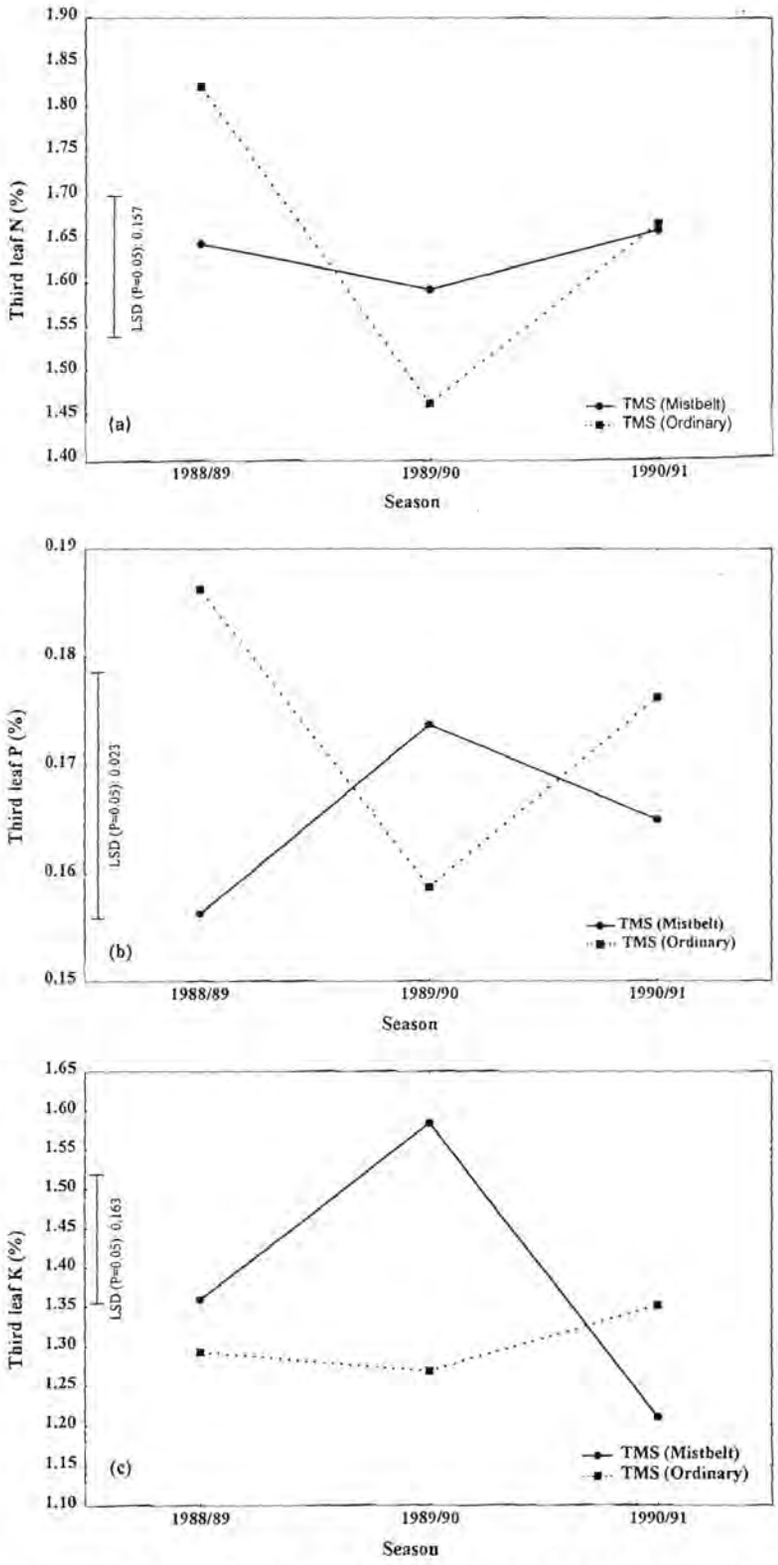


Figure 2.3. Leaf nutrient values associated with the samples collected from the Midlands estate during the 1988/89 (including 1987/1988), 1989/90 and 1990/91 seasons.

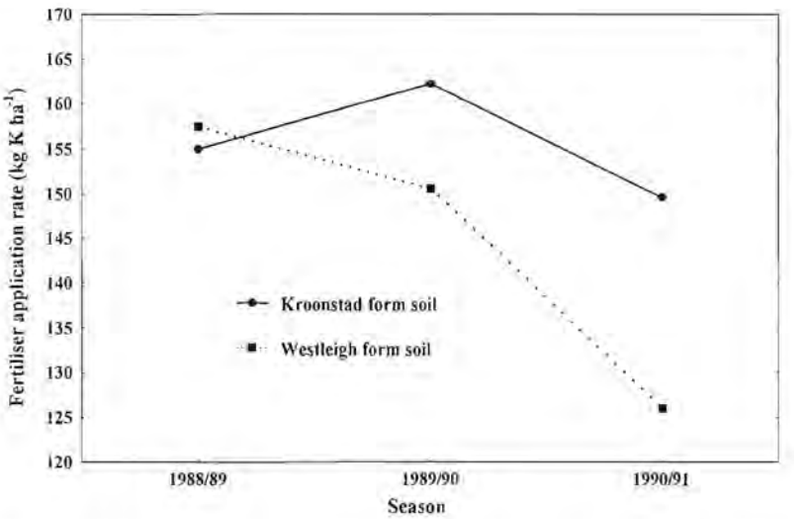


Figure 2.4. Potassium fertiliser application rates on the different soil forms on the Zululand estate over the period 1988/89 to 1990/91.

2.4. Conclusions

The following conclusions were drawn:

- The examples and case studies in this chapter provided substantial evidence that the below average rainfall that occurred in much of the South African sugar industry in the summer of 1989/90 had an effect on the nutrient (N, P and K) content of third leaf samples submitted to the FAS laboratory for analysis.
- This qualitative / semi quantitative approach was important in highlighting this effect and providing a fairly good foundation for assuming that the low N and P values associated with many of the 1989/90 samples were the result of moisture stress effects rather than nutrient deficiencies *per se*.
- Moisture stress appeared to cause variable responses in third leaf K values.
- In the absence of an index of moisture stress, growers should always be encouraged not to sample if moisture stress effects are suspected. However in view of the limits in time and age of cane at sampling, this strategy, which is indeed sensible, has severely curtailed the use of one of the most effective 'tools'

available for making informed decisions about fertiliser management in the South African sugar industry.

- The interactive effects indicated in the second case study highlighted the importance of soil type when leaf sampling. Sugarcane grown on sandy shallow soils will be more easily affected by moisture stress than cane grown on deep loamy type soils.
- A more rigorous evaluation of the effect of moisture stress on leaf analysis and the major content of sugarcane was deemed warranted.

Chapter 3.

The interaction between moisture stress, plant growth and the nitrogen content of sugarcane.

3.1. Introduction

The interaction between water supply and plant growth has received much attention in sugarcane production over the years. Such studies have mostly been aimed at identifying production constraints, making yield predictions, optimising irrigation scheduling, etc (Thompson, 1988; Inman-Bamber, 1991; Inman-Bamber, 1995; van Antwerpen *et al*, 1996). In particular, it is well documented that moisture stress influences dry matter accumulation and sucrose yield in sugarcane (Inman-Bamber and de Jager, 1988(a). As expected, reports indicate that moisture stress also affects growth or plant extension (Bull and Glaziou, 1975; Inman-Bamber and de Jager, 1988(b)), and by association, attributes such as leaf area index (LAI), stomatal resistance and leaf water potential (Inman-Bamber, 1986).

As indicated in Chapter 1 of this dissertation, much progress has also been made in the use of leaf analysis for diagnostic, advisory and nutrient trend purposes in various sugar producing countries. Although it is also recognised that plant water relations may affect plant nutrition (Marschner, 1993), little quantitative information is available regarding the effect of moisture stress on the nutrient content of sugarcane leaves. This interaction and the understanding thereof is fundamentally important in the interpretation of leaf analysis data, especially in countries such as South Africa where foliar testing is considered to be extremely important in assessing the adequacy of fertiliser applications.

Based on available evidence from the South African sugar industry that moisture stress conditions were indeed affecting leaf nutrient values (Chapter 2), this investigation was aimed at quantifying the interaction between moisture stress, plant

growth and the nitrogen content of adequately fertilised sugarcane grown in pots under semi-controlled conditions. This was done by utilising some of the methodology and concepts that have been developed and used by agronomists in the more conventional water supply/ plant growth studies mentioned earlier.

3.2. Materials and methods

Three sugarcane plants of uniform height (about 150mm) that had been pre-germinated from single budded setts of variety NCo376 were planted into each of thirty two 80 litre containers (Figure 3.1) that were filled with 90 kg of air-dried red loamy sand topsoil (Table 3.1). The soil had previously been passed through a 5mm mesh and fertilised with limestone ammonium nitrate (LAN), single super phosphate and potassium chloride at rates equivalent to 140 kg N ha^{-1}, 20 kg P ha^{-1} and 100 kg K ha^{-1} respectively.

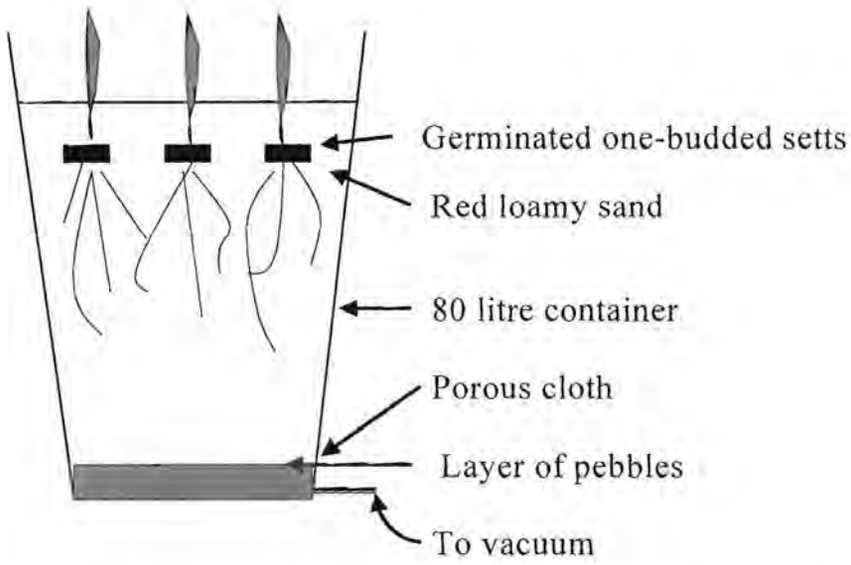


Figure 3.1. A diagrammatic representation of the planted containers used in the investigation.

Table 3.1. Some chemical and physical characteristics of the soil used in this investigation.

Soil[a] form	Soil[a] series	Clay (%)	Organic matter (%)	Soil $pH_{(water)}$	Extr $P_{(Truog}$[b]$)$ ($mg\ kg^{-1}$)	Exchangeable cations (1M Ammomium Acetate) ($mg\ kg^{-1}$)			
						K	Ca	Mg	Na
Hutton	Clansthal	11	1.8	8.4	>80	92	1375	48	59
FAS critical values					31	112	150	25	460[c]

[a] (Macvicar *et al*, 1977))
[b] 0.1M H_2SO_4
[c] sodic conditions may be suspected if values are greater than 460mg kg-1

The planted containers (Plate 1) were placed under an automatically controlled rain-shelter at the SASEX Central Field Station near Umhlanga Rocks (Plate 2) and regularly watered (every 2 to 3 days) to predetermined masses to ensure that soil moisture content was maintained at field capacity. Each container was attached to an individual vacuum trap (Figure 3.1 and Plate 3) at a pressure differential of about 10kPa (to simulate moisture content of sands at field capacity). Any water that seeped into the pebble layer was again transferred back into the top of that container. Once the cane had reached three months of age, moisture stress treatments were applied according to the experimental design details given below.

3.2.1. Experimental design

The experimental design was a 4 X 4 (moisture stress X harvest date) factorial trial with two replications.

The moisture stress treatments were as follows:

- **Unstressed**: the soils continued to be kept at field moisture capacity until harvest.
- **Stressed (early)**: water was withheld from day 90 after planting.
- **Stressed (late)**: water was withheld from day 100 after planting.

- **Stress/relief**: water was withheld from day 90 after planting, but stress was relieved after day 110 by watering the soil to field moisture capacity once more.

The harvest dates were as follows:

- approximately **100** days after planting
- approximately **110** days after planting
- approximately **120** days after planting
- approximately **130** days after planting

3.2.2. Experimental details

From day 85 after planting, plant growth was assessed by utilising hourly plant extension rate (HPER) data collected from a system of growth transducers (variable resistors) linked to a Campbell Scientific CR10 data logger (Figure 3.2) as developed by the Agronomy and General Services Departments at SASEX (Anon, 1993). In this system the spindle of a plant is attached with cord to a variable resistor which is spring loaded (Figure 3.2 and Plate 4). As growth occurs the variable resistor turns creating a potential difference that can be read and recorded. With previous calibration of these growth transducers, the potential differences over set time periods can be transformed back into distance values that correspond to plant growth or plant extension rate (PER) eg mm plant growth per hour. In this particular experiment the potential difference values were read every minute, averaged over a five-minute period, summed every hour and then recorded. The attachment to the spindle was changed to a different fully growing shoot every few days to ensure that measurement was associated with youngest expanding leaf in all cases. HPER values were calculated from the potential difference values according to the equation:

PER (mm/hr) = potential difference over an hour period (mV) x (a/b)

where a is a factor relating mV to distance

and b is a calibration factor relating length to actual plant growth (NG Inman-Bamber – pers. comm.).

For the purposes of this experiment:

a = 0.25

b = 0.71.

Daily plant extension rate (DPER) was calculated by summing the hourly extension rate values over a twenty-four hour period. In particular, mean DPER values were calculated for five specific three-day periods ie. prior to the imposition of the moisture stress treatments (period 1: 13 weeks after planting) and again prior to each harvest (periods 2, 3, 4 and 5). Periods 2, 3, 4 and 5 were respectively within weeks 14, 15, 16 and 18 after planting.

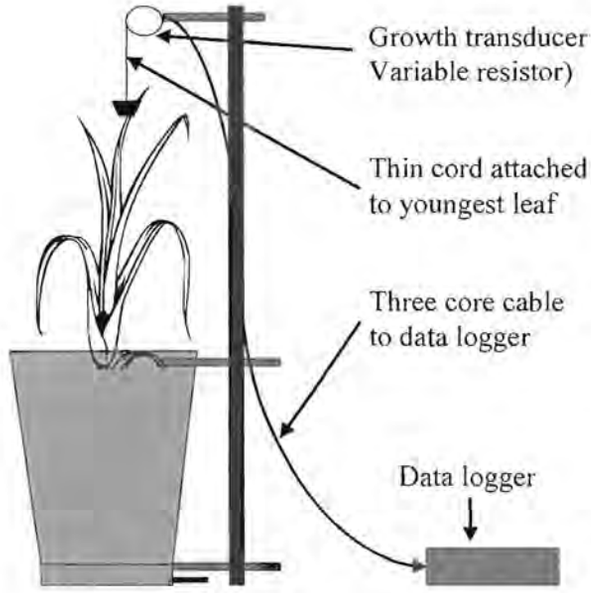


Figure 3.2. Measurement of hourly plant extension rate - diagram showing the connection of a growth transducer to the youngest leaf of one of the plants in a container.

At harvest, the three plants (consisting of numerous shoots/tillers) from each container were destructively sampled by removing all plant material to ground level. The shoots were divided up into three groups according to size (small, medium and large). The area associated with the green leaves of six representative shoots (two large, two medium and two small) was measured and the mean area per shoot size was calculated. The LAI of the plants from each container was estimated from the sum of the total green leaf area per size class (Plates 5 and 6). LAI values were expressed as area of green leaf (m^2) per surface area of soil in each container (m^2). The harvested plants were then partitioned (Plate 8) and composite samples were formed according to their leaf and sheath number. The third leaf samples were further partitioned into sub-samples. These consisted of the middle 200mm section of each lamina with midribs removed (as usual for third leaf sample), the removed midribs, the lower sections of the laminae (from sheath to the removed 200mm section) and the top sections of the laminae (above the 200mm section). Any trash and stalk present were placed in separate samples. These composite component samples were weighed, dried in a forced draught oven at 70°C and re-weighed. The plant material was finely ground and passed through a 0.5mm perforated screen and then chemically analysed according to standard procedures in the FAS laboratory (Appendix A). Dry matter yield per container was obtained by summing the dry masses of all the component parts and expressed as t ha^{-1}.

The soil moisture content was calculated for each pot at harvest according to the equation:

Soil moisture content (%) = $((m_f - (m_c + m_s + m_{wp})) \times 100)/m_s$

where:

mf is the final mass of the container plus total contents
m_c is the original mass of the container (including additionals)
m_s is the original mass of the air-dried soil added to the container
m_{wp} is the total wet mass of the harvested plants.

3.3. Results and discussion

3.3.1. Effect of moisture stress on plant growth

To assess whether plant growth patterns and rates were similar within the trial prior to the imposition of the moisture stress treatments (as indicated above), a 'snap-shot' of the hourly growth was obtained. This was done by plotting mean HPER (mm hr^{-1}) values against time for a single day in the week before the imposition of stress treatments (Figure 3.3). Although the growth patterns observed on this day were all similar to each other (Figure 3.3(a)) and typical of those for sugarcane (Anon, 1994a), the mean hourly growth rate in the plants associated with the future treatment 3 were significantly higher than the growth rates associated with the other future treatments (Figure 3.3(b)). As expected there were significant differences in plant growth during the 24 hour period (Figure 3.3(c)) with the maximum plant extension rate occurring between 4:00pm (1600hours) and 6:00pm (1800hours).

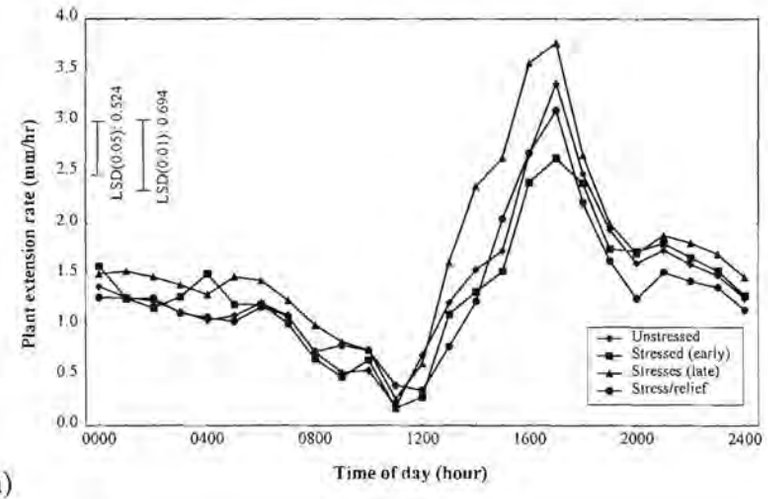
The difference in mean HPER value between the future "stressed (late)" treatment and the other future treatments was not considered a problem, as in all cases the mean daily plant extension rate over a three day period prior to the imposition of the moisture stress treatments (Table 3.2) was above 20mm per day (the current norm for minimum growth required for leaf sampling).

Table 3.2. Mean daily plant extension rate over the three-day period just prior to the imposition of moisture stress treatments.

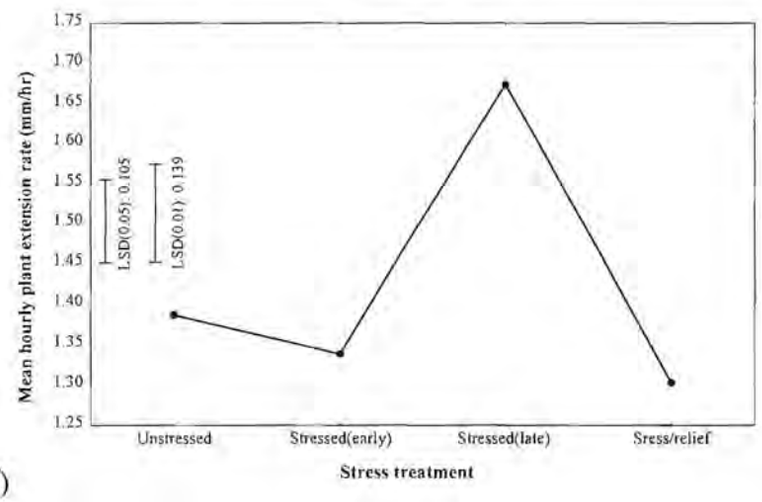
Future moisture stress treatment	Mean DPER (mm day^{-1})
Unstressed	37.0
Stressed (early)	36.5
Stressed (late)	46.4
Stress/relief	38.8
SE	2.1
LSD (0.05)	6.5
LSD (0.01)	9.2

While DPER values were further used in preference to the HPER values for gauging plant growth during the trial, the 24 hour 'snap-shot' assessments of the hourly plant growth patterns associated with the different moisture stress treatments were still undertaken prior to each harvest. These are shown in Appendix B.

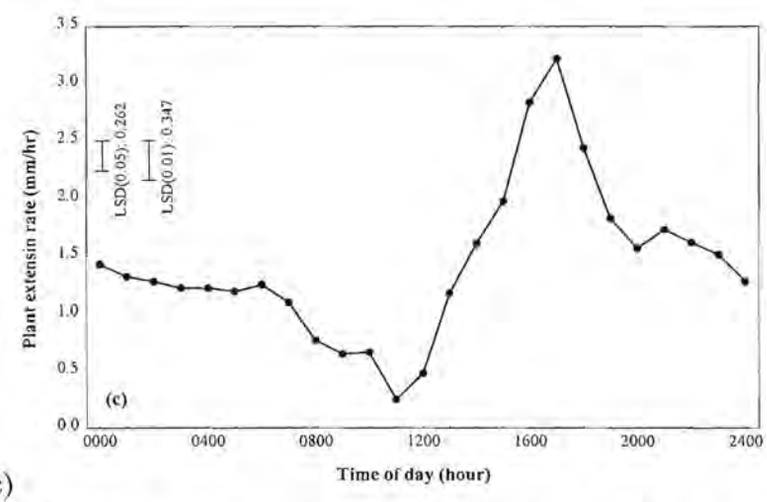
As the soil used in this investigation had a relatively low water holding capacity (12%), moisture stress effects on plant growth soon became visually apparent within the treatments in which irrigation was withheld. These negative responses to stress were reflected in mean DPER values recorded over three-day periods prior to each harvest (Figure 3.4) and showed a significant interaction between moisture stress treatment and time (as successive harvest periods). Whereas plant growth continued to be maintained at values above 20mm per day in the unstressed plants (Figure 3.4), it significantly declined in both the early and late stressed treatments. With stress/relief, growth first declined as water was withheld but then increased again to above 20mm per day once the moisture stress was relieved. It should be noted however, that the apparent decline in HPER associated with the unstressed conditions (treatment 1) during period 3 (prior to the second harvest: week 15 after planting) was thought to be the result of temporary mild moisture stress effects.



(a)



(b)



(c)

Figure 3.3. Plant growth pattern and rates associated with the sugarcane in a single day prior to the imposition of stress: HPER plotted against time (a), mean hourly plant extension rates in plants associated with future treatments (b) and mean plant extension rates for the 24hr period (c).

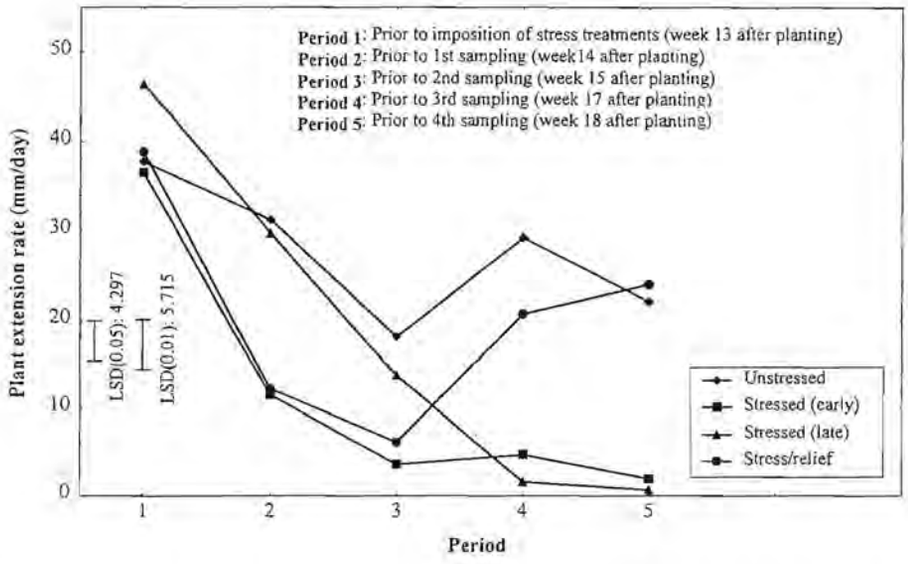


Figure 3.4. Mean plant extension rate values associated with the various moisture stress treatments with time.

As expected the soil moisture content progressively decreased with time in relation to both the early and late stressed treatments, and also in the stress/relief treatment prior to re-irrigation at 100 days after planting (Figure 3.5). Highly significant differences in mean soil moisture content values were noted between the unstressed and early stressed conditions across the full thirty-day period (100 to 130 days after planting). Although the soil moisture contents of the unstressed and late stressed treatments were similar on day 100, they had become significantly different by day 110. This difference continued to widen with time. While no significant difference in soil moisture content existed between the early stressed and stress/relief treatments up until day 100, irrigation improved the water status of the stress/relief treatment to that of the unstressed treatment. The apparent increase in soil moisture content with unstressed conditions was thought to be associated with root mass accumulation rather than moisture accumulation *per se*.

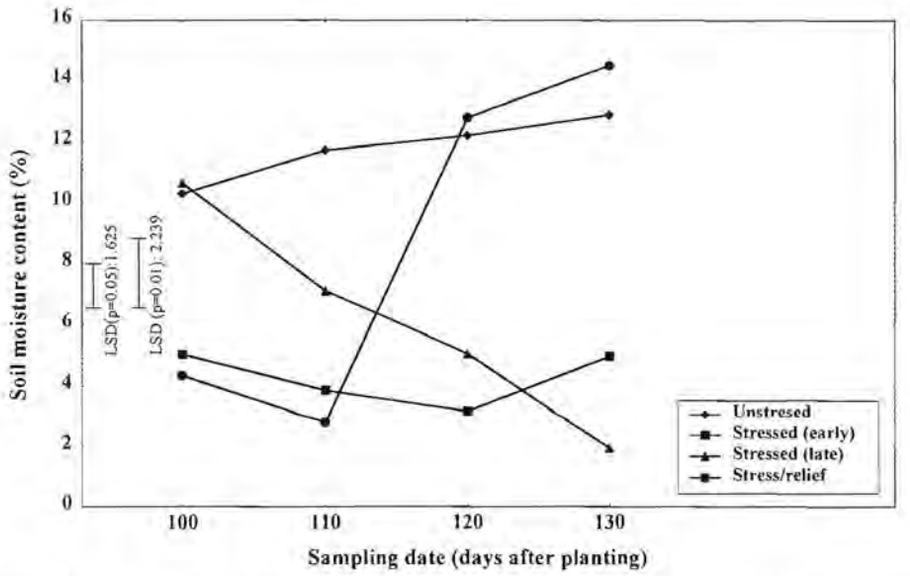


Figure 3.5. Mean soil moisture content values associated with the unstressed, stressed and stress/relief treatments over the thirty-day harvest period of the investigation.

3.3.2. Effect of moisture stress on dry matter production and LAI

The dry matter yield data (t ha^{-1}) indicated that there was a significant interaction between the moisture stress and sampling date (Figure 3.6). Although no significant difference existed between the dry matter yield at the 100- day harvest, the yield associated with the future 'stressed (late)' treatment was somewhat higher than that of the other treatments. This reflected the higher growth rate that was previously identified in this treatment. Whereas the dry matter yield associated with the unstressed conditions increased with time, it remained essentially static throughout the 30-day period with early stress. Despite the imposition of stress at the later stage, in treatment 3, the initial unstressed conditions appeared to allow some initial increase in dry matter production. There was evidence of dry matter yield improvement associated with the stress/relief treatment once the plants had been re-watered (subsequent to day 110 after planting).

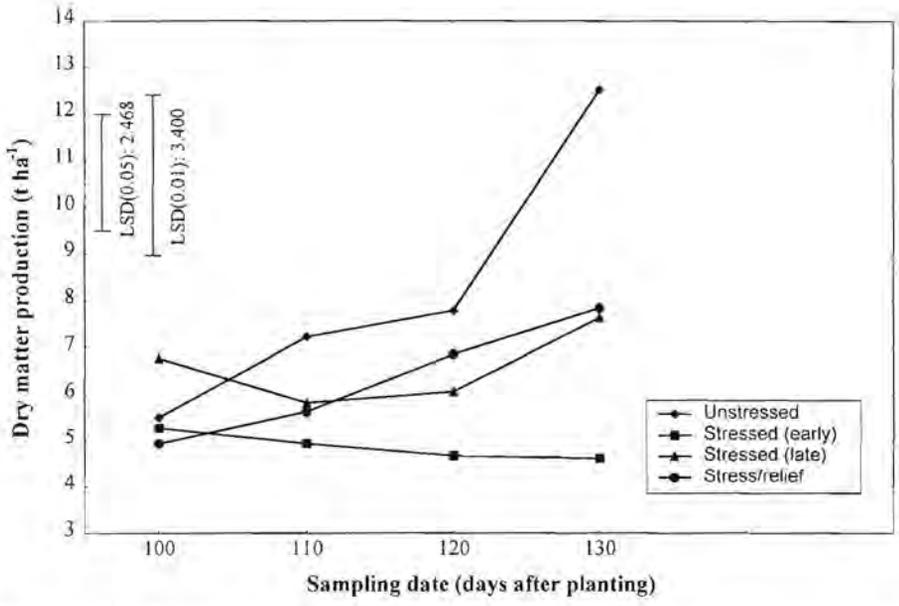


Figure 3.6. Interactive effect between moisture stress and sampling date on dry matter yield.

Similarly, the interactive effect between moisture stress and sampling date was reflected in the calculated LAI values (Figure 3.7). While the mean LAI value increased significantly with time in the unstressed cane, it declined with time under all three moisture stress conditions. With stress/relief, the mean LAI value increased once water was re-applied. The highly significant difference between the mean LAI values of the moisture stressed cane (early and late) and that of the stress/relief treatment was evidence of the recovery in growth once moisture stress was relieved. The fact that the highest LAI values recorded in this study were above those normally quoted for sugarcane at full canopy ie. 5.1 to 5.6 (Gosnell, 1967; Haslam and Allison, 1985) and 6.4 (Thompson, 1988), was attributed to the fact that the plants were grown in containers (with restricted soil surface areas) rather than field conditions. However, the relative LAI values in this instance was considered more important than the absolute values associated with the various treatments.

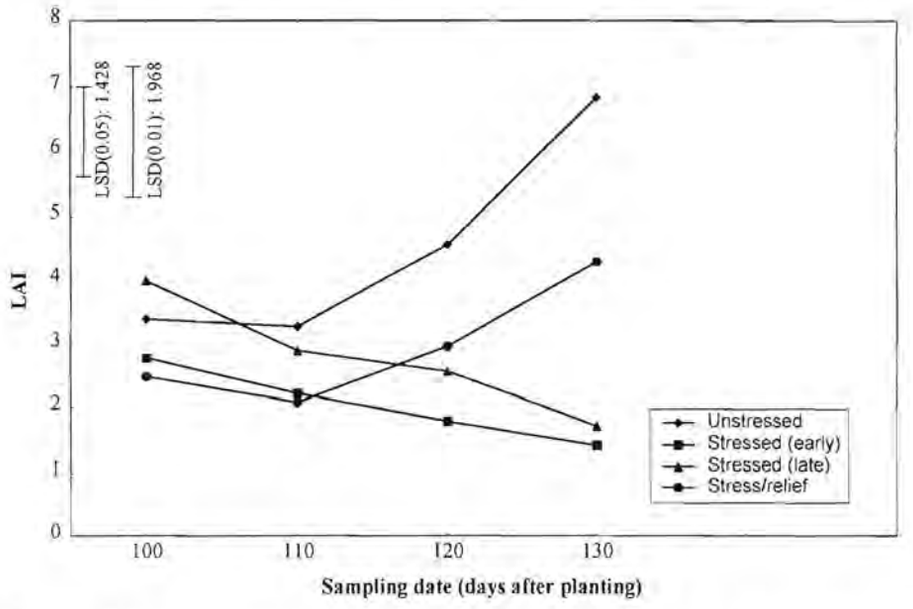


Figure 3.7. Interactive effect between moisture stress and sampling date on LAI.

3.3.3. Effect of moisture stress on plant N

Although analysis data for all the plant components harvested were available, only those associated with the spindle and first to six leaves (lamina and sheath) were used in the statistical analysis. The main effects associated with the analysis of variance (four moisture stress treatments, four harvest dates and 15 plant parts) indicated that significant differences existed between mean plant N values (%) associated with the various moisture stress treatments, sampling dates and the various plant parts (Table 3.3). In particular it was noted that, as expected, leaf N values declined with increasing leaf number, as did sheath N values. Separation of the third leaf samples into the different components (Table 3.3) showed that the N values of the mid 200mm section of the lamina (L3La) was similar to that of the lower section of the lamina (L3R). The midrib samples had the lowest N value of the third leaf components.

Furthermore, in relation to the plant N content, there was a significant interaction between moisture stress treatment and sampling date (Figure 3.8). By day 100 after planting (10 days after the imposition of the moisture stress treatments)

significant difference in plant N content existed between the unstressed cane and the cane associated with the stressed (early) and stress/relief treatments. By day 110 after planting (20 days after the imposition of the moisture stress treatments) these difference had become even more apparent. Whereas the plant N content of the cane associated with treatment 3 (stressed (late)) declined rapidly after irrigation was withheld, it increased considerably in the cane associated with treatment 4 (stress/relief) once re-watering had occurred. By day 130 after planting (40 days after the imposition of the moisture stress treatments, and 20 days after the stress was relieved in treatment 4), significant differences no longer existed between the plant N content of the cane associated with the unstressed and stress/relief treatments. As expected a gradual decline in the plant N content of the cane (as seen in the unstressed cane) occurred with time (Figure 3.8).

Table 3.3. Effects of moisture stress, sampling date and plant parts on plant N content.

Moisture stress	Plant N (%)	Sampling date (days after planting)	Plant N (%)	Plant parts (spindle, leaf and sheath numbers)	Plant N (%)
Unstressed	1.56	100	1.49	Sp[1]	1.84
Stressed (early)	1.20	110	1.35	L[2]1	1.76
Stressed (late)	1.36	120	1.33	L2	1.73
Stress/relief	1.30	130	1.23	L3La[3]	2.09
				L3M[4]	1.15
				L3R[5]	1.96
				L3T[6]	1.69
				L4	1.56
				L5	1.31
				L6	1.32
				S[7]2	1.32
				S3	0.91
				S4	0.67
				S5	0.58
				S6	0.47
SE	0.03		0.03		0.06
LSD (0.05)	0.09		0.09		0.17
LSD (0.01)	0.11		0.11		0.22

[1]Sp = spindle; [2]L = Leaf; [3]La = lamina (mid 200mm section with midrib removed); [4]M = midrib (from 200mm section); [5]R = lower section of leaf (between the sheath and the 200mm section); [6]T = top section of the leaf (between the 200mm section and the tip); [7]S = sheath

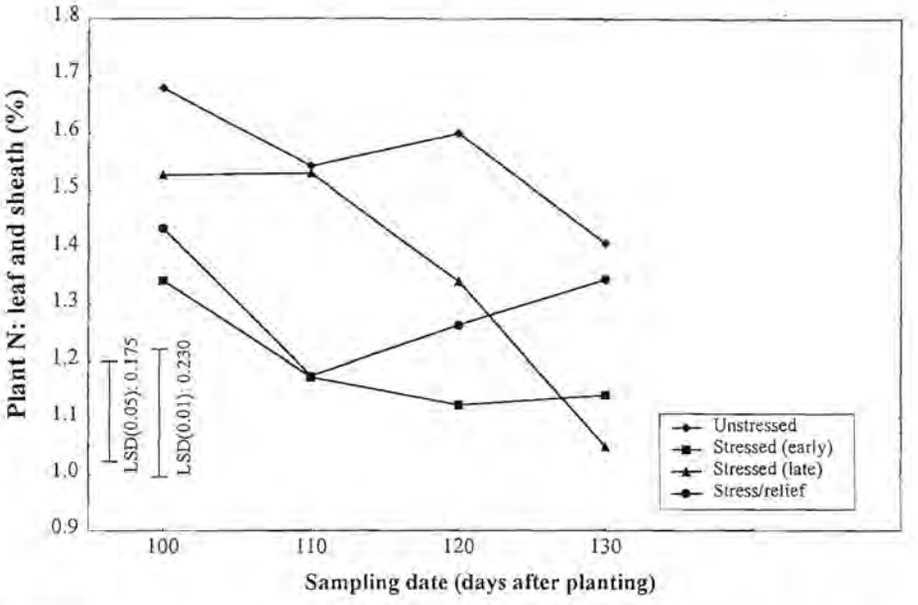


Figure 3.8. Interactive effect of moisture stress and sampling date on plant N content.

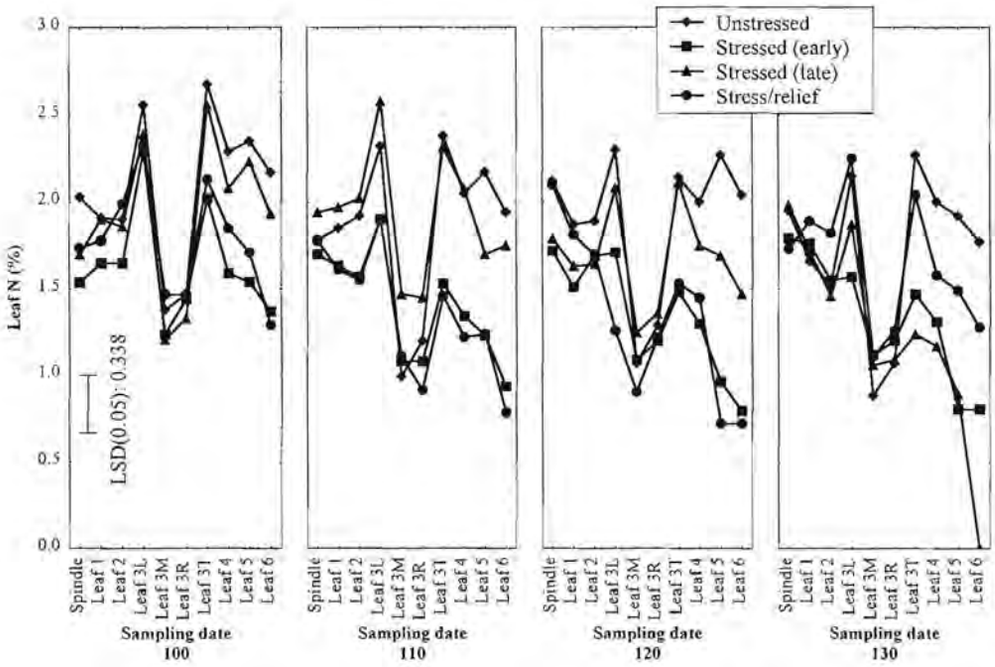
Apart from the interactive effect on the N content of the entire plant, these general moisture stress X sampling date effects were also generally apparent in the various plant components (Figure 3.9).

When the data relating to the third leaf in particular was considered, it was found that the moisture stress treatments and date of sampling had a significant effect on leaf N content (Table 3.4). However, the third leaf N content did not appear to be as sensitive to changes in moisture availability as was seen with PER for instance. Although the mean third leaf N value declined with time when irrigation was withheld (Figure 3.10), the differences in third leaf N content between the unstressed and stressed (early) treatment were not statistically significant. In the case of the stress/relief treatment, the mean third leaf N value associated with the cane harvested at 120 days after planting (30 days after the imposition of moisture stress) was significantly different from that of the unstressed cane. This was despite the re-irrigation that had occurred from day 110 after planting ie. ten days before sampling. However by day 130 after planting, ie. twenty days after re-watering, the mean third leaf N value of the cane associated with the stress/relief

treatment had increased significantly. At this stage the third leaf N value was not dissimilar to that of the unstressed cane (Figure 3.10).

Table 3.4. Effects of moisture stress and sampling date on third leaf N content.

Moisture stress	Plant N (%)	Sampling date (days after planting)	Plant N (%)
Unstressed	2.33	100	2.39
Stressed (early)	1.87	110	2.18
Stressed (late)	2.23	120	1.84
Stress/relief	1.94	130	1.96
SE	0.11		0.11
LSD (0.05)	0.35		0.35
LSD (0.01)	0.48		0.48



La = lamina (mid 200mm section with midrib removed); M = midrib (from 200mm section); R = lower section of leaf (between the sheath and the 200mm section); T = top section of the leaf (between the 200mm section and the tip).

Figure 3.9. Effect of moisture stress and sampling date on N content of various leaf samples.

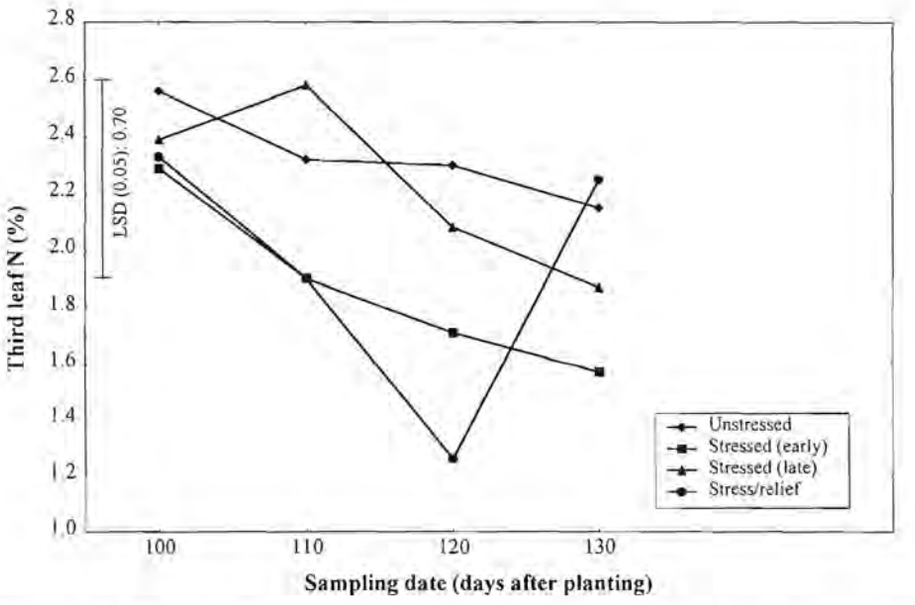


Figure 3.10. The effect of moisture stress and sampling date on third leaf N values.

3.4. Conclusions

The following conclusions were drawn:

- The initial HPER and DPER values (prior to the application of the moisture stress treatments) indicated that growth was characteristic of cane not subject to moisture stress.
- As expected, the visual responses to moisture stress were reflected in plant growth and soil water content.
- DPER values were a good indicator of the intensity of moisture stress. However such measurement is not practically possible in commercial fields of sugarcane.
- Decreased dry matter yield and LAI values confirmed the existence of moisture stress when irrigation was withheld.
- The increase in DPER, soil water content, LAI and dry matter yield after moisture stress relief was indicative of plant recovery with re-watering.

- The accelerated decline in plant N values with time when water was withheld (early and late) was the result of moisture stress effects rather than an N deficiency *per se*.
- The decline in plant N (total leaf and sheath) with moisture stress and its recovery with re-irrigation indicated that total plant N was directly associated with water supply.
- Evidence suggested that a delay in recovery in the third leaf N values occurred when moisture stress was relieved. Redistribution of N probably occurred to the younger plant tissue (spindle, first and second leaves) rather than to existing fully expanded leaves once moisture stress was relieved.
- The delay in recovery of third leaf N values compared to the more rapid recovery in plant growth has important implications for interpreting leaf analysis data. In particular, it is considered insufficient to base recommendations for suitable leaf sampling periods solely on minimum plant growth rate.
- This initial assessment indicated that further work was warranted in assessing
  - the interaction between moisture stress, plant growth and the N content of sugarcane with different N fertiliser rates,
  - the interaction between moisture stress and other plant macro nutrients,
  - the availability of practical and 'easy to use' moisture stress indices,

  and comparing the interaction between moisture stress, plant growth and nutrient content of sugarcane in different varieties.

Chapter 4.

Leaf N values as affected by nitrogen application rate and moisture stress

4.1 Introduction

In view of the results and conclusions of Chapter 3, it was considered important to investigate further the interaction between moisture stress and nitrogen. In the first experiment (Trial 1), an adequate rate of N was applied to all containers. Any changes in leaf N value were therefore associated with relatively high plant N status. In order to understand the effect of moisture stress on sugarcane with sub-optimal N levels, it was deemed necessary to investigate the moisture stress effect on plant N (and third leaf N values in particular) when N was limiting.

This investigation was therefore aimed at quantifying the interaction between moisture stress, plant growth and leaf N values when N was applied below the recommended rate. This was done by comparing the effects of moisture stress and stress/relief conditions on sugarcane grown in containers that had received either adequate or below recommended rates of N.

4.2 Procedure

The data discussed here were obtained from a further experiment (Trial 2) conducted in semi-controlled conditions beneath the automatically controlled rain-shelter at the SASEX Central Field Station near Umhlanga Rocks. The establishment procedure was identical to that described in Section 3.2 (Trial 1), with the following important exceptions:

- Nitrogen was applied at two rates (as indicated in the experimental design details given below).

- The moisture stress treatments were applied once the cane had reached four and a half months of age. The cane was grown in the cooler part of the year (April to September) to ensure that moisture stress effects did not occur too rapidly. Under such conditions, a longer growing season was thought necessary prior to the imposition of the moisture stress treatments. In addition, the sampling period was also extended.

4.2.1. Experimental design

The experimental design was a 2 X 2 X 4 (N application rate X moisture stress X harvest date) randomised pot trial with two replications. However because three factors were involved in this study and sampling date was considered to be a 'dependent' rather than 'independent' variable, the dry matter yield, LAI and third leaf nutrient data were analysed according to a split-plot design where N application rates and moisture stress treatments were regarded as 'whole-plot' factors and the four harvest dates were considered to be the 'sub-plot' factors. In terms of the nutrient data relation to the partitioned plants, the analysis of variance was conducted according to a standard randomised block design.

The N application rates were as follows:

- **Full N rate**: equivalent to 120 kg N ha^{-1} (as would be recommended by the SASEX fertiliser advisory service).
- **Half N rate**: equivalent to 60 kg N ha^{-1} (half of that recommended by the SASEX fertiliser advisory service).

The stress treatments were as follows:

- **Unstressed**: soil was kept at field capacity throughout the whole experiment by periodic watering (every two to three days).
- **Stress/relief**: water was withheld from day 140 after planting, but stress was relieved after day 165 from planting (at the third harvest date) by watering the

soil to field moisture capacity and maintaining it as such by periodic watering (every two to three days).

The sampling dates were as follows:

- Approximately **145** days after planting.
- Approximately **155** days after planting.
- Approximately **165** days after planting.
- Approximately **175** days after planting.

4.2.2. Experimental details

As it was found in Trial 1 that the level of moisture stress in these particular experimental circumstances were reflected in a range of possible measurements, HPER and DPER were not determined as the procedures could easily be substituted by more easily obtained determinations at the time of harvest. The use of soil moisture content and LAI were considered sufficiently suitable to quantify the level of moisture stress.

The sampling (harvest) procedure used was the same as that described in Chapter 3. As such, all plants from the relevant containers were destructively sampled and partitioned according to their leaf and sheath number, and any stalk if present. Third leaf samples were also again partitioned into four sub-samples (middle 200mm sections of each lamina with midribs removed (L3La), the removed midribs (L3M), the lower sections of the laminae (L3R) and the top sections of the laminae (L3T)). LAI, dry matter yield and soil moisture content values were determined as before (Section 3.2.2).

4.3 Results and discussion

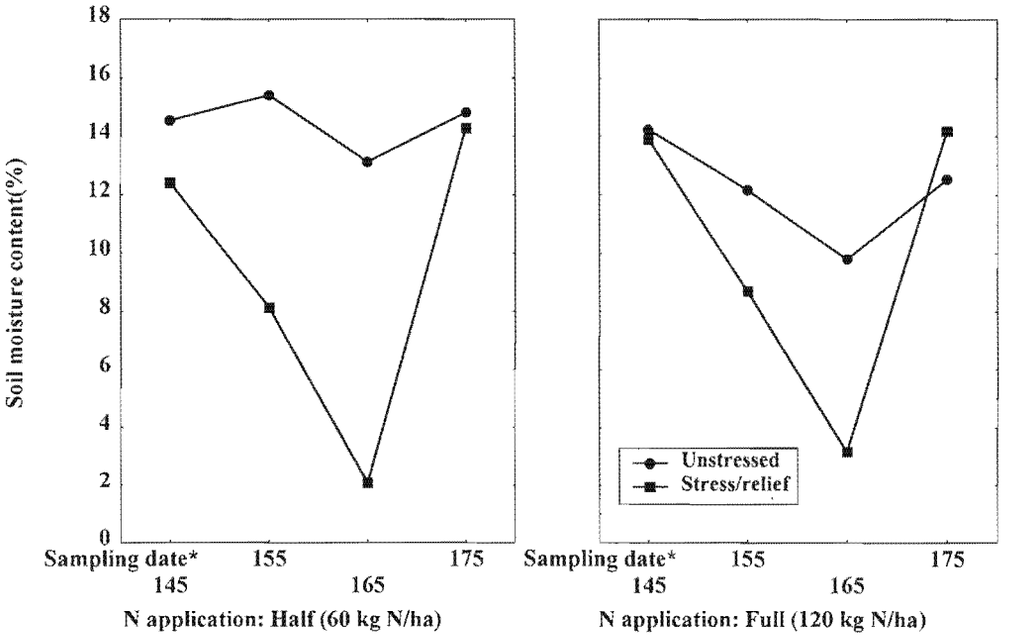
4.3.1. Effect of moisture stress treatments on soil moisture content and LAI

The soil moisture content determined at harvest (Figure 4.1) indicated that moisture stress effects existed within the containers when water was withheld

(stress/relief treatment). Moisture stress was relieved with irrigation after day 165. Although it was intended that there would be continuous moisture stress free conditions in the unstressed treatments, the soil moisture content values indicated that some moisture stress occurred in the containers associated with the Full N treatment (Figure 4.1). This occurred as the containers were watered to set masses and no allowance was made for the relatively large amounts of biomass associated with the Full N treatment after the first harvest.

Nitrogen application rate had a highly significant effect on LAI (Table 4.1). As expected an increase in N application rate resulted in an increased mean LAI value. The imposition of moisture stress had the opposite effect and caused a highly significant decline in LAI values (Table 4.1). In addition, there was a significant interactive effect on LAI between N application rate and moisture stress treatment (Table 4.1). Whereas with the high N application rate, the imposition of moisture stress severely affected LAI, the decrease in LAI associated with the lower N rate was not significant.

Although the analysis of variance did not indicate an interactive effect between N application rate, moisture stress treatment and harvest date on LAI, it is useful to take note of the trends (Figure 4.2) that can be identified in the interaction table. With the stress/relief treatment, mean LAI values declined for both rates of N applied and increased again once the plants had been re-irrigated. LAI values generally increased with time in the 'unstressed' conditions.

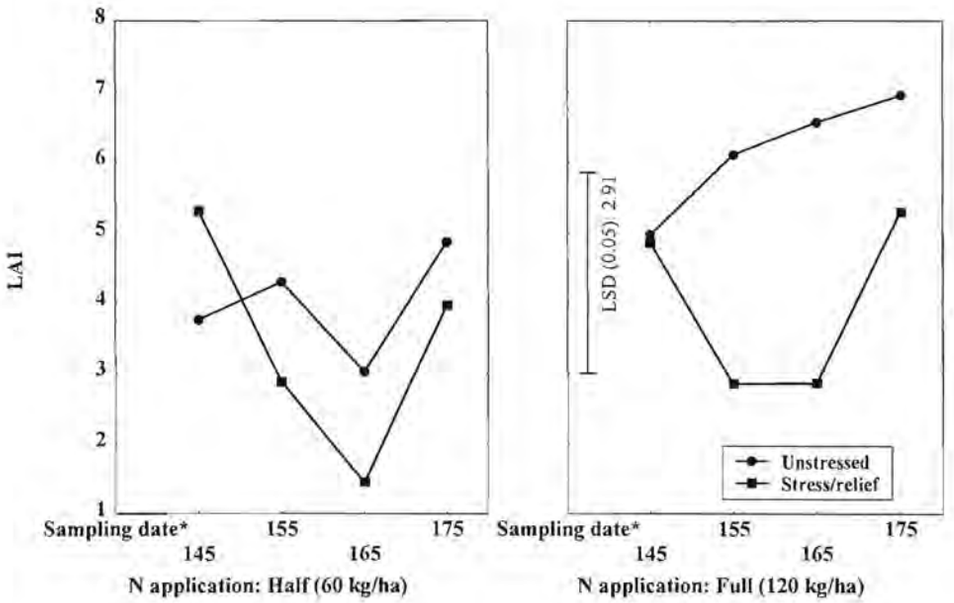


* days after planting

Figure 4.1. Soil moisture content (%) associated with the moisture stress treatments at the various sampling dates.

Table 4.1. Effect of N application rate and moisture stress on LAI values.

Leaf area index (LAI)			
Main effects			
Moisture stress		Nitrogen applied	
Unstressed	5.070	Half rate	3.696
Stress/relief	3.684	Full rate	5.058
SE	0.175	SE	0.175
LSD (0.05)	0.687	LSD (0.05)	0.687
LSD (0.01)	1.138	LSD (0.01)	1.138
Interactive effect			
Moisture stress treatment	Nitrogen applied		
	Half rate	Full rate	
Unstressed	3.989	6.151	
Stress/relief	3.404	3.965	
SE: 0.247 LSD (0.05): 0.971 LSD (0.01): 1.610			



* days after planting

Figure 4.2. LAI values associated with the moisture stress treatments at the various sampling dates when N was applied at two different rates.

4.3.2. Dry matter production as influenced by moisture stress and N application rate.

Unlike LAI, which is related to the number of green leaves present, dry matter yield is associated with the total bio-mass production. As a result, it was found that the dry matter yield increased with time over the 30-day sampling period (Figure 4.3). However the dry matter yield was dependent on the interaction between N applied and the moisture stress treatment (Figure 4.4). In terms of the unstressed treatment, a significant difference in dry matter yield (as calculated over the whole sampling period) was apparent between the cane fertilised at the lower (60 kg N ha^{-1}) and higher rate (120 kg N ha^{-1}). However, in relation to the stress/relief treatment, no significant differences in yield occurred over the 30-day sampling period. Although the analysis of variance did not indicate a three-way interaction (between N application rate, moisture stress treatment and harvest date), it was considered useful to take note of the trends (Figure 4.5) that were identified. These contributed to a better understanding of the full implication of the stress/relief treatment. As the moisture stress treatment contained both a

moisture-stress and a stress-relief component, mean values tended to mask the changes that occurred over the 30 day period. As such, the increased accumulation of dry matter associated with the unstressed conditions and the high N application rate (as seen in Figure 4.4) were more clearly identified in the three way interaction (Figure 4.5).

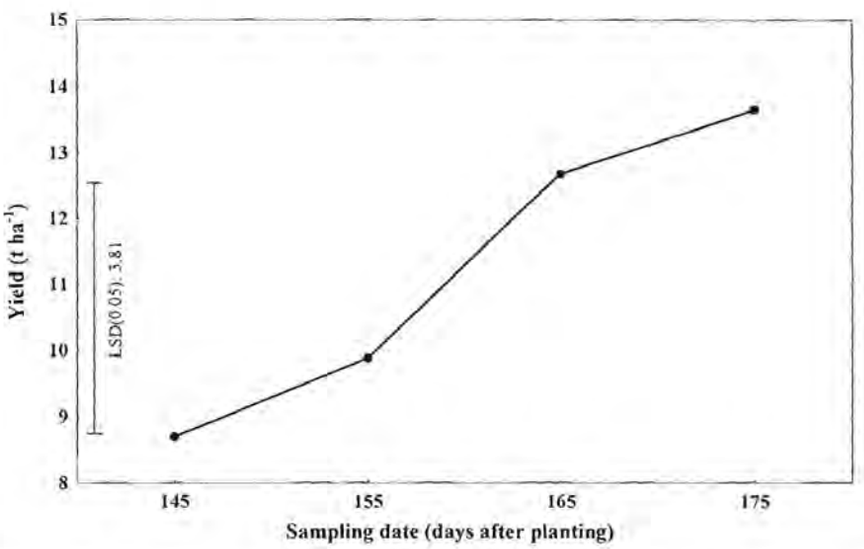


Figure 4.3. Dry matter yield as measured over the 30-day sampling period.

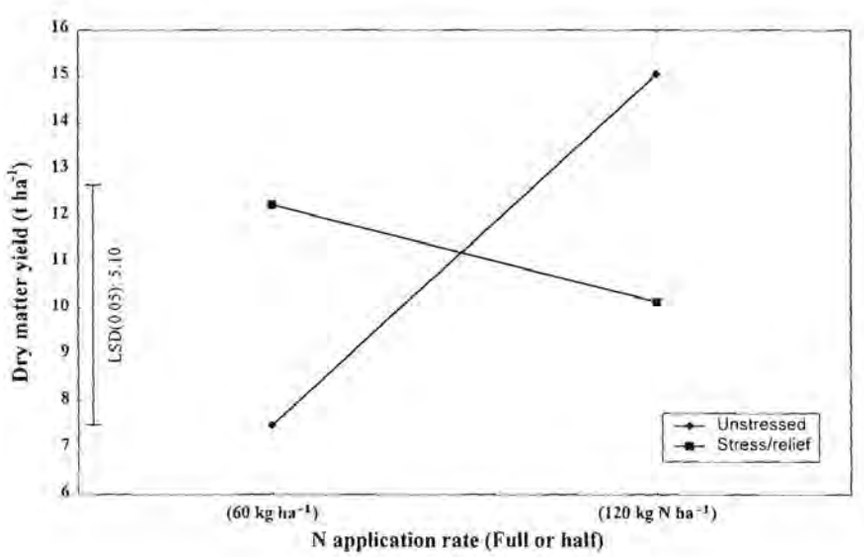


Figure 4.4. The interactive effect of N application rate and moisture stress treatment on dry matter yield.

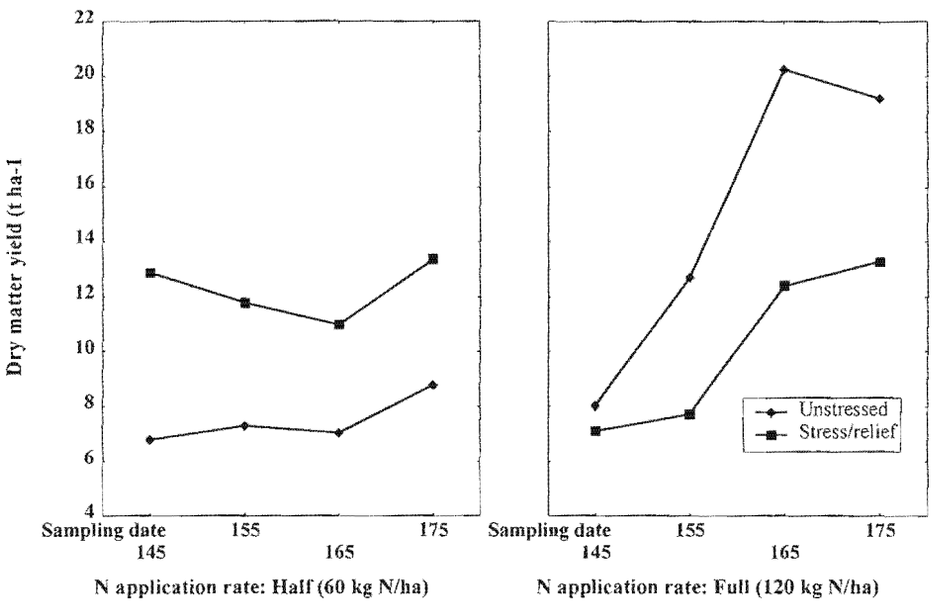


Figure 4.5. The influence of N application rate and moisture stress treatment on dry matter yield over the 30-day sampling period.

4.3.3. The interactive effect of N application rate and moisture stress on the N content of the partitioned plants.

Although the chemical analysis data for all plant components was available, only those associated with the spindle, the first to sixth leaves (lamina and sheath) and stalk (if present) were used in the statistical analysis. The main effects associated with the analysis of variance (two rates of N, two moisture stress treatments, four harvest dates and 16 plant parts) indicated that highly significant differences existed between the mean plant N (%) values within the various 'treatments' (Table 4.2). As expected, the mean plant N(%) for the cane fertilised at half the recommended rate was significantly lower than that of the cane fertilised at the full recommended rate. Similarly, the mean plant N (%) for the unstressed cane was significantly higher than that of the stressed cane, and on average, plant N (%) declined during the sampling period. Significant differences also existed in plant N(%) associated with the various plant parts. Apart from the stalk, which had the highest N (%) value, the spindle had the next highest mean N(%) value. When the whole leaf was considered, N was found to decline with increasing leaf

number. In relation to the third leaf, which was partitioned, the mid 200mm section with the midrib removed (L3La) was similar to that of the top section of the leaf (L3T), but significantly higher that that of the midrib (L3M) and the lower section of the leaf (L3R). Sheath N values declined with increasing sheath numbers.

Table 4.2. Effects of moisture stress, sampling date and plant parts on plant N content.

N application rate	Plant N (%)	Moisture stress	Plant N (%)	Sampling date (days after planting)	Plant N (%)	Plant parts (spindle, leaf and sheath numbers, stalk)	Plant N (%)
Half (60 kg N ha^{-1})	1.088	Unstressed	1.258	145	1.44	Sp[1]	1.647
		Stress/relief	1.072	155	1.26	L[2]1	1.351
Full (120 kg N ha^{-1})	1.242			165	0.87	L2	1.282
				175	1.09	L3La[3]	1.631
						L3M[4]	0.863
						L3R[5]	0.938
						L3T[6]	1.590
						L4	1.347
						L5	1.223
						L6	1.106
						S[7]2	1.100
						S3	0.798
						S4	0.544
						S5	0.544
						S6	0.490
						St[8]	2.182
SE	0.017	SE	0.017	SE	0.025	SE	0.049
LSD (0.05)	0.048	LSD (0.05)	0.048	LSD (0.05)	0.068	LSD (0.05)	0.136
LSD (0.01)	0.063	LSD (0.01)	0.063	LSD (0.01)	0.090	LSD (0.01)	0.179

[1]Sp = spindle; [2]L = Leaf; [3]La = lamina (mid 200mm section with midrib removed); [4]M = midrib (from 200mm section); [5]R = lower section of leaf (between the sheath and the 200mm section); [6]T = top section of the leaf (between the 200mm section and the tip); [7]S = sheath; [8]St = stalk.

In considering the whole plant, the analysis of variance indicated that there were two significant three-way interactions ie. N application rate x stress treatment x sampling date (Figure 4.6) and stress treatment x sampling date x plant parts (Figure 4.7).

In relation to the N application rate x stress treatment x sampling date, it was found that under unstressed conditions the whole plant mean N(%) values were not significantly different from each other for much of the sampling period. However, in relation to the moisture stress/relief treatment, the plant N (%) associated with the lower N application rate was already significantly lower than that of the higher N application rate on day 145 after planting (about one week after irrigation was withheld). Although the plant N(%) associated with the higher N application rate declined with the increase in moisture stress with time (Figure 4.1), a significant increase in plant N(%) occurred once the moisture stress was relieved (Figure 4.6). However, in the case of the lower N application rate, no recovery in plant N (%) was noted.

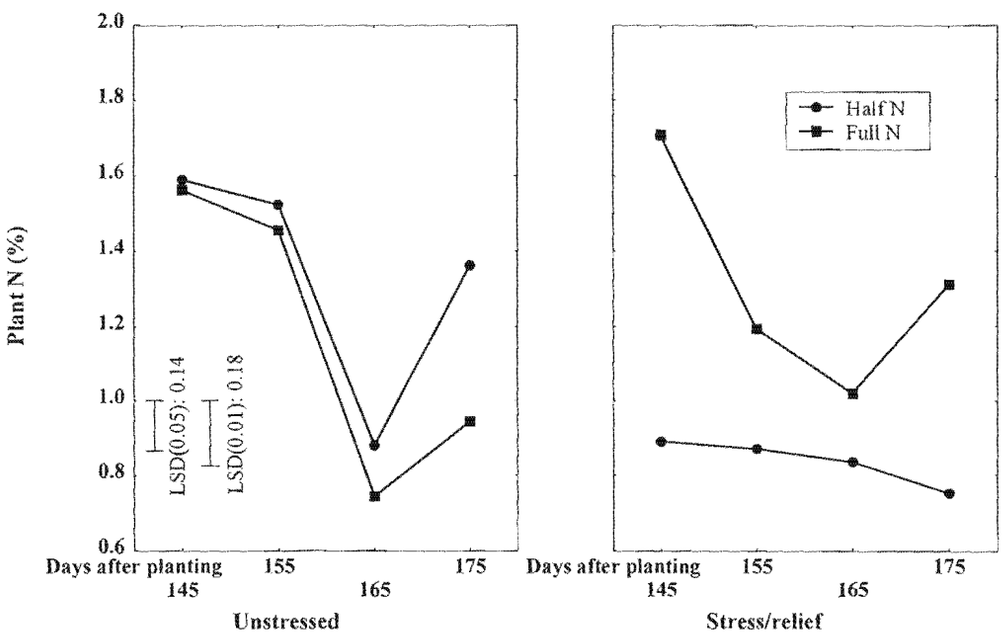
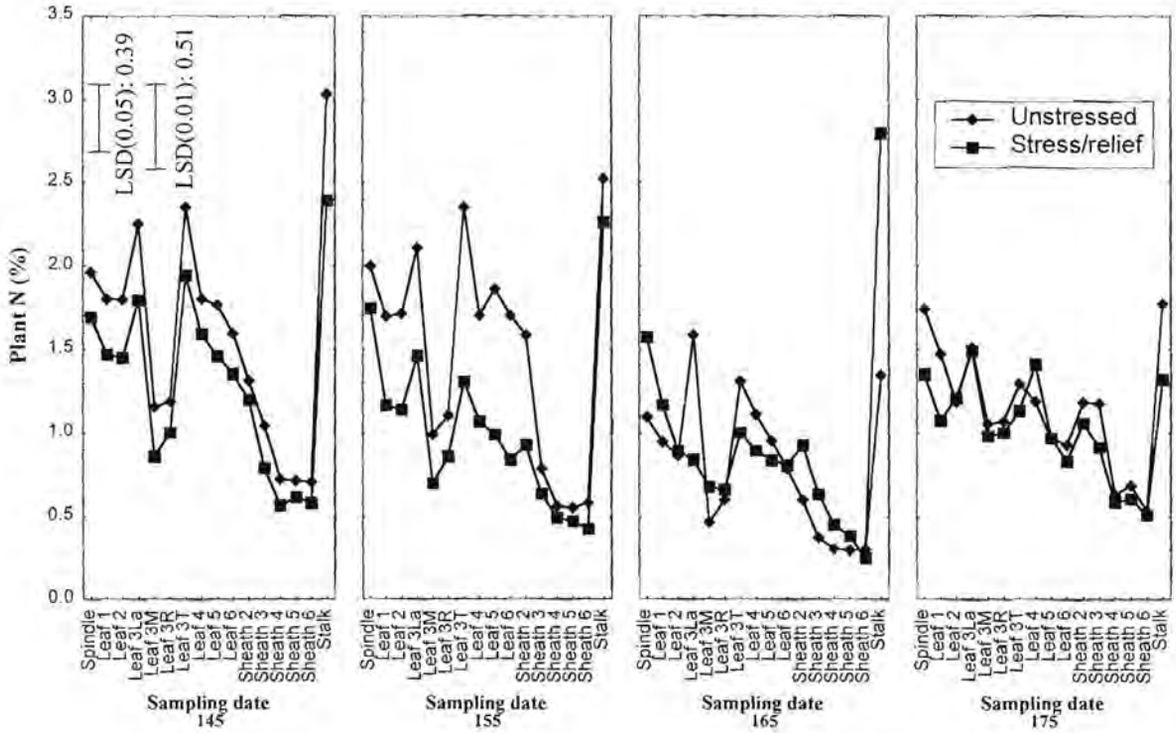


Figure 4.6. Interactive effect of N application rate and sampling date on plant N(%).

In relation to the stress treatment x sampling date x plant part interaction, it was observed that by day 155 after planting (about two weeks after the imposition of the moisture stress), the N contents of most of the leaves of the stressed cane were

significantly lower than that of the unstressed cane. Once moisture stress had been relieved (day 175 after planting and ten days after re-watering), the plant N (%) content of the cane associated with the unstressed and stress/relieved treatments was very similar (Figure 4.7). The fact that the plant N content of the unstressed cane on day 165 was significantly lower than the N values on the previous and subsequent sampling dates (Figures 4.6 and 4.7), offered further evidence to suggest that the 'unstressed' cane was indeed affected by temporary moisture stress on the third sampling date (Figure 4.1). The substantial increase in stalk N with stress (Day 165 after planting) and its subsequent decline once stress was relieved, suggested that under stress conditions, N from the leaves is absorbed into the stalk and then redistributed to the spindle and young leaves once stress has dissipated.



La = lamina (mid 200mm section with midrib removed); M = midrib (from 200mm section); R = lower section of leaf (between the sheath and the 200mm section); T = top section of the leaf (between the 200mm section and the tip).

Figure 4.7. Effect of moisture stress and sampling date on the N content of the various plant parts.

4.3.4. The interactive effect of N application rate and moisture stress on the third leaf N(%) values.

Third leaf N values were affected by both N application rate and moisture stress and by their interaction (Figure 4.8). The fact that the mean third leaf N values associated with the higher N application rate did not significantly decrease with the stress/relief treatment indicated that substantial improvement in the individual third leaf N values must have occurred once the moisture stress was relieved. In comparison, the mean third leaf N value associated with the lower N application rate failed to recover after the moisture stress dissipated. This resulted in the significant difference in mean third leaf N values observed between the sugarcane that had received the higher and lower N fertiliser applications within stress/relief treatments.

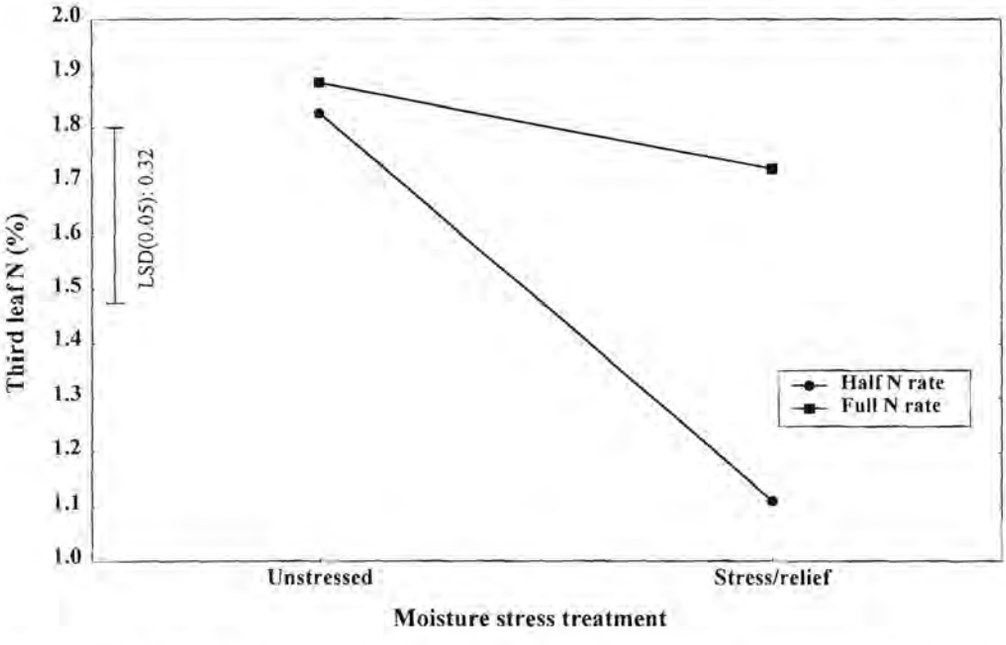


Figure 4.8. The interactive effect of N application rate and moisture stress on third leaf N values.

Third leaf N values were also affected by the interaction between moisture stress and the sampling date (Figure 4.9). The consistently significant differences that

were observed between the third leaf N values associated with the unstressed and stress/relief treatment during the period of moisture stress (day 145 to day 165 after planting) disappeared once the moisture stress was relieved.

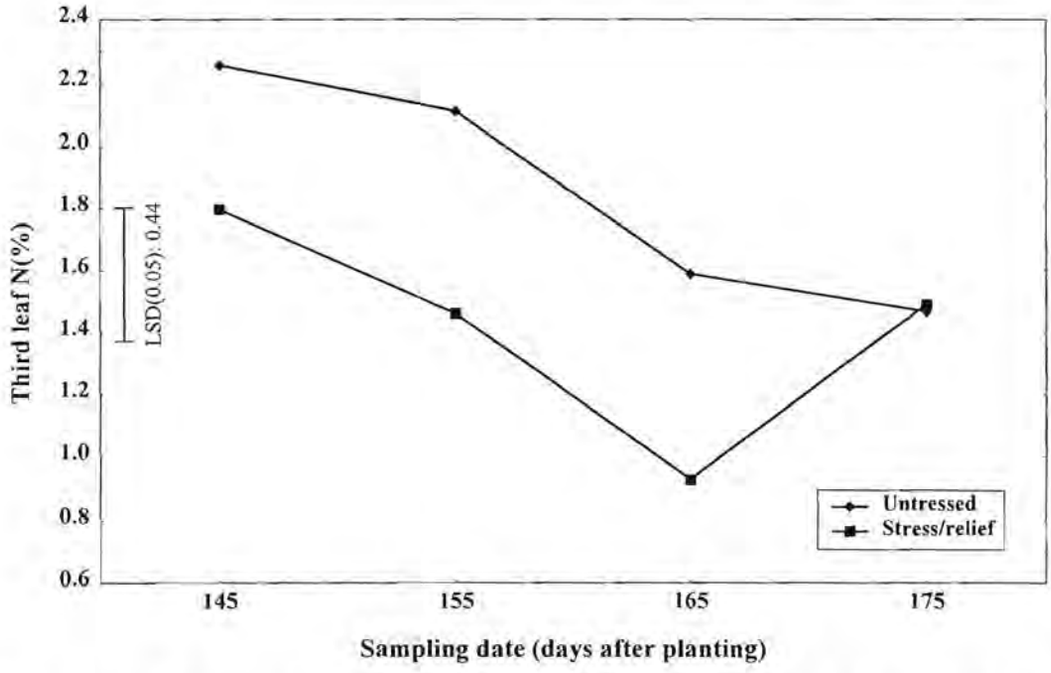


Figure 4.9. The effect of moisture stress and sampling date on third leaf N values

As indicated previously in this chapter, the full implication of the stress-relief treatment, which consisted of both a moisture stress and stress/relief component, appeared to be hidden in some circumstances within the calculated mean values. Although the three-way interaction associated with the third leaf N values was not significant, it more clearly illustrated both the recovery in N status with the higher N application rate and the lack of recovery with the lower N application once the moisture stress had been relieved (Figure 4.10).

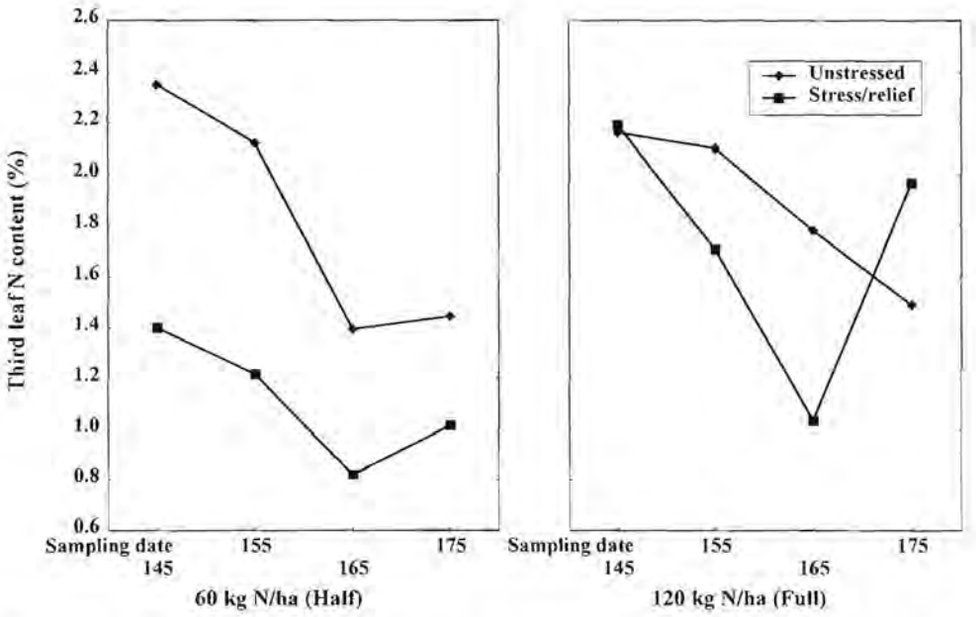


Figure 4.10. The influence of N application rate and moisture stress on third leaf N values.

4.4 Conclusions

The following conclusions were drawn:

- As before, plant N and third leaf N values declined under moisture stress conditions. When N was adequately supplied, a recovery in plant N occurred once the moisture stress conditions were relieved. However, when N was limiting, significant increases in plant N did not occur when the stress was relieved.
- Third leaf N (%) values fairly closely reflected the overall N status of the partitioned plants in relation to the higher and lower N application rates under both unstressed and stress/relief conditions.
- The fact that stalk N increased under conditions of moisture stress has important implications for the interpretation of N in cane stalk or juice for advisory or diagnostic purposes. Scientists investigating such aspects would need to consider the use of a suitable moisture stress index in this regard as well.
- The interactions identified in this study confirm that third leaf N values need to be used in association with some type of moisture stress index to ensure that low N values resulting from moisture stress effects are not confused with low leaf N values *per se*.

Chapter 5.

The interaction between the other leaf macro-nutrients (P and K) and moisture stress

5.1. Introduction

Despite general recognition that moisture stress conditions affect plant nutrient content, it appears that the limited investigations aimed at quantifying these effects have been directed more towards N than any of the other nutrients (Gosnell and Long, 1971). However, based largely on anecdotal evidence from the FAS data-base, it would seem that, as with N, a decline in leaf P values may also occur under conditions of moisture stress. In addition, variable responses in third leaf K content have been reported under conditions of moisture stress (Evans, 1961). Although such effects on leaf P and K are supported by the semi-quantitative analysis described in Chapter 2 (of this dissertation) and the conclusion drawn by Gosnell and Long (1971), data from experiments performed under controlled conditions do not appear to be comprehensive nor easily available. In attempting to interpret leaf analysis data better, it was also speculated that generally higher levels of ash in cane at South African sugar mills during the drought conditions in 1994 (Lionnet, 1995) may have been the result of increased K uptake by sugarcane under conditions of moisture stress. This hypothesis was based on the fact that potassium in plants provides an osmotic gradient to facilitate water uptake (Marschner, 1993). As such, increased K uptake by the crop under conditions of moisture stress was thought to be a possible mechanism by which plants could extract additional water from drought-affected soils.

In light of the paucity of definitive data relating to the interaction between plant P and K and moisture stress, this investigation was aimed at using the pot trials described in Chapters 3 and 4 to quantify this interaction when both P and K were adequately supplied.

5.2. Materials and methods

The data discussed here were obtained from the two experiments (Trials 1 and 2) described in Chapters 3 and 4. In both cases nutrients other than N (P, K, Ca and Mg) were either adequately present in the soil (Table 3.1) or applied at rates recommended by the SASEX fertiliser advisory service. In both cases single super phosphate and potassium chloride were applied at rates of 20 kg P ha^{-1} and 100 kg K ha^{-1} respectively. The experimental design and details were as described in Chapters 3 and 4 of this dissertation (Sections 3.2.1, 3.2.2, 4.2.1 and 4.2.2). In relation to Trial 2, only the data relating to the full N application rate (120 kg N ha^{-1}) was used in the statistical analysis of the total and partitioned plant P and K data. However, the full data-set was utilised in the statistical analysis of the third leaf data.

5.3. Results and discussion

The effect of moisture stress on soil moisture content and plant growth in both the trials was well established and described in Chapters 3 and 4 and will therefore not be discussed here. It should be viewed as given that moisture stress affected plant growth, LAI and wet and dry matter production and that recovery occurred with re-watering. The effect of moisture stress and stress/relief on plant P and K under adequately fertilised conditions is described below. As with the assessment of plant N, only the data associated with the spindle and first six leaves (lamina and sheath) were used in the statistical analysis although the balance of data relating to the other plant components was available. As stalk was present at the times of sampling in Trial 2, data included 'stalk' values.

5.3.1 Effect of moisture stress on plant P (Trial 1)

The main effects associated with the analysis of variance (four moisture stress treatments, four sampling dates and 15 plant parts) indicated that highly significant differences existed between the mean total plant P (%) values within the various 'treatments'. This related to the moisture stress treatments, sampling date and plant parts (Table 5.1). The total plant P in the unstressed treatment was significantly different ($P<0.01$) from that of the other treatments (stress related),

and total P in the stress/relief treatment was significantly different from that of the comparable stressed (early) treatments. This indicated that some recovery in plant P occurred when moisture stress was relieved. As with N, plant P also declined with increasing leaf and sheath number. The P content of the sheaths was generally lower than that of their associated lamina, although the leaf and sheath P values of the second leaf were similar. In relation to the partitioned third leaf, the P content of the mid 200mm section with the midrib removed (L3La) was similar to that of the top section of the leaf (L3T), but significantly higher than that of the midrib (L3M) and the lower section of the leaf (L3R).

Table 5.1. Effects of moisture stress, sampling date and plant parts on plant P content.

Moisture stress	Plant P (%)	Sampling date (days after planting)	Plant P (%)	Plant parts (spindle, leaf and sheath numbers)	Plant P (%)
Unstressed	0.222	100	0.197	Sp[1]	0.295
Stressed (early)	0.126	110	0.172	L[2]1	0.240
Stressed (late)	0.186	120	0.175	L2	0.206
Stress/relief	0.167	130	0.159	L3La[3]	0.231
				L3M[4]	0.154
				L3R[5]	0.168
				L3T[6]	0.215
				L4	0.192
				L5	0.162
				L6	0.128
				S[7]2	0.218
				S3	0.141
				S4	0.106
				S5	0.093
				S6	0.082
SE	0.004		0.004		0.009
LSD (0.05)	0.012		0.012		0.023
LSD (0.01)	0.016		0.016		0.031

[1]Sp = spindle; [2]L = Leaf; [3]La = lamina (mid 200mm section with midrib removed); [4]M = midrib (from 200mm section); [5]R = lower section of leaf (between the sheath and the 200mm section); [6]T = top section of the leaf (between the 200mm section and the tip); [7]S = sheath

In considering the whole plant, the analysis of variance indicated that there were two significant two-way interactions ie. stress treatment x sampling date (Figure 5.1) and stress treatment by plant part (Figure 5.2). It was found that there was little difference in total plant P between the cane associated with the unstressed and stressed (late) conditions at the first (100 days after planting) and second (110 days after planting) date – both being essentially unstressed (Figure 5.1). However, significant differences in total plant P developed from the third sampling date (day 120 after planting), as the stress conditions became more manifest. The total plant P associated with the stressed (early) conditions was significantly different ($P<0.01$) by the first sampling date (day 100 after planting and 10 days after water was withheld) and remained so for the full sampling period. In relation to the stress/relief treatment, total plant P declined with moisture stress but showed little improvement after moisture stress was relieved by re-watering.

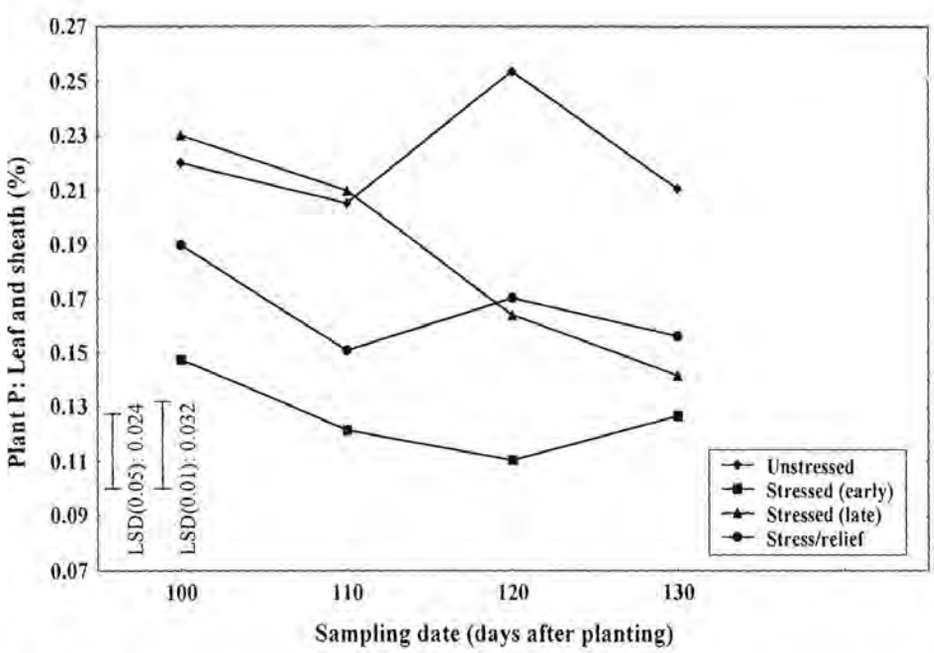
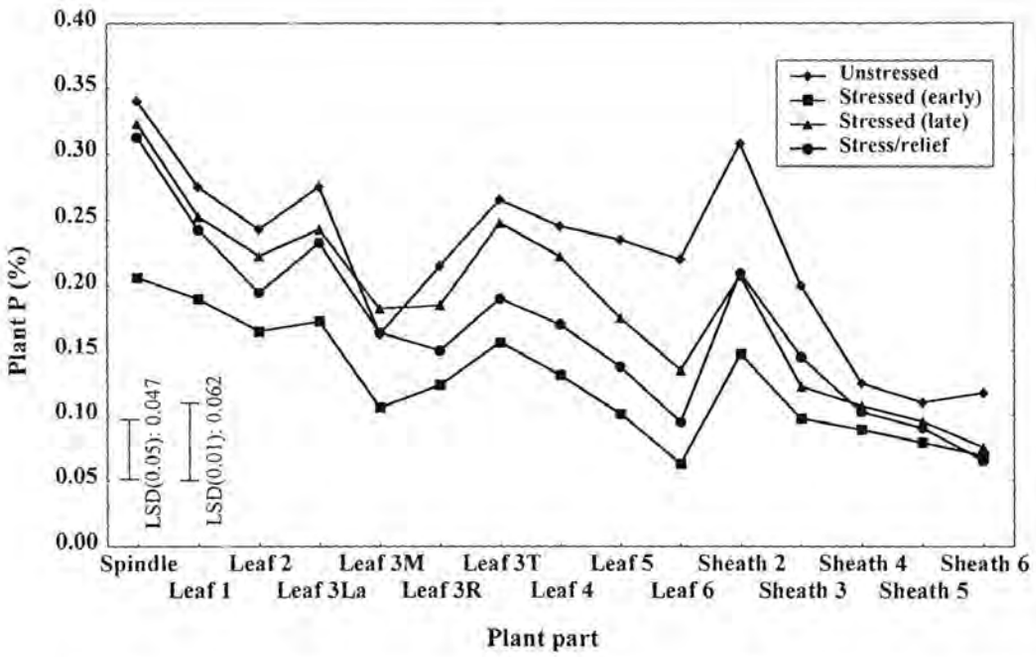


Figure 5.1. The interactive effect of moisture stress and sampling date on total plant P.

In relation to the stress treatment x plant part interaction (Figure 5.2), it was found that mean P values associated with the different plant parts (over the four harvest

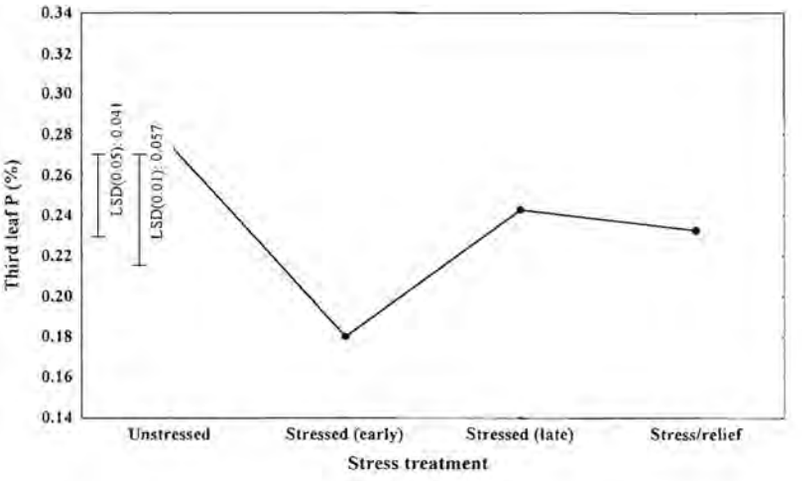
dates) under conditions of moisture stress (stressed (early)) were generally significantly lower than those of the corresponding unstressed conditions. The exceptions (not significantly different) were those associated with the lower sheaths (sheaths 4, 5 and 6) and the midrib of the third leaves (Leaf 3M). The mean plant P values of the various plant parts associated with the stressed (late) were similar but lower than those associated with the unstressed conditions. In relation to the stress/relief treatment the leaf P values were generally higher (not always significant) than those associated with the stressed (early) treatment. However, this did indicate that some recovery in P values occurred, especially in the actively growing part of the plant (spindle, and lamina and sheath of leaf one).



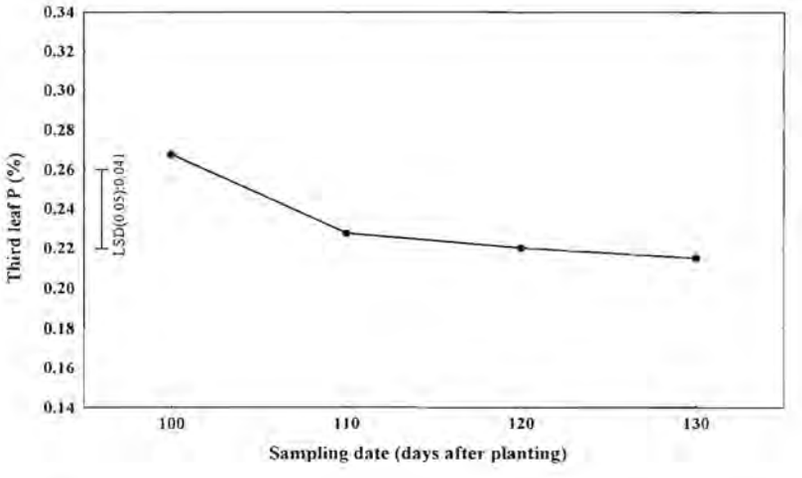
La = lamina (mid 200mm section with midrib removed); M = midrib (from 200mm section); R = lower section of leaf (between the sheath and the 200mm section); T = top section of the leaf (between the 200mm section and the tip).

Figure 5.2. The influence of moisture stress on the P content of the different plant parts under the various moisture stress conditions (treatments).

When only the third leaf samples were considered, it was found that both moisture stress and sampling date had a significant effect on leaf P content (Figure 5.3).



(a)



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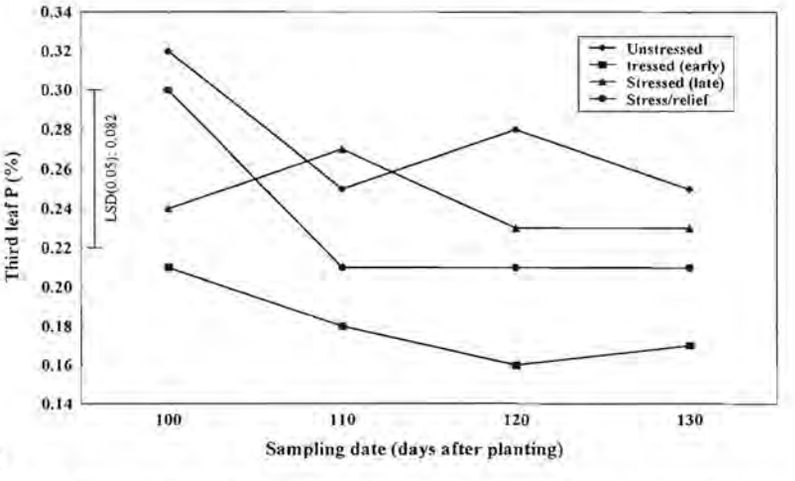


(c.)

Figure 5.3. Third leaf P (%) as affected by moisture stress treatment (a), sampling date (b) and the interaction between sampling date and moisture stress treatment (c).

The main effects showed that in terms of moisture stress, the mean third leaf P value associated with the stressed (early) treatment was significantly lower than that of the other treatments, which were not significantly different from each other (Figure 5.3(a)). This trend may be explained in terms of the moisture stress conditions that were more manifest in the stressed (early) conditions compared to that of the stressed (late) treatment (Figure 3.5). The mean leaf P value associated with the stress/relief treatment was an average value that reflected both stressed and unstressed conditions. Mean third leaf P declined with sampling date (Figure 5.3(b)), but remained above the critical leaf value (Table 1.1).

In terms of the interactive effect of moisture stress treatment and sampling date (Figure 5.3(c)), the mean third leaf P value associated with the stressed (early) treatment was already significantly lower than that of the unstressed treatment by day 100 after planting (10 days after water was withheld), and remained so for the full sampling period. Initially (at 100 days after planting) the third leaf P values associated with the stress/relief treatment was significantly higher than those of the stressed (early) treatment. However it declined thereafter to values that were consistently similar to those associated with stressed (early) treatment for the rest of the sampling period. Improvement in the third leaf P status with stress relief was not apparent, as in the case of mean the third leaf N value (Figure 3.10).

5.3.2 Effect of moisture stress on plant P (Trial 2)

The main effects associated with the analysis of variance (two moisture stress treatments, four sampling dates and 16 plant parts) indicated that highly significant differences existed between the mean total plant P (%) values (covering all the plant parts considered) within all the 'treatments' (Table 5.2). The total plant P in the unstressed cane was significantly different (P0.01) from that of the stress/relief treatment. In the case of "sampling date", it was found that the mean plant P values were generally not dissimilar to each other, the exception being the total plant P associated with the third sampling date (165 days after planting and 25 days after water was withheld). This unusual decline and

subsequent improvement in mean total plant P was thought to be associated with possible uncharacteristically dry conditions (as described in Chapter 4) that may have affected the cane on the third sampling date. In relation to the plant parts, P declined with increasing leaf and sheath number. With the exception of the second leaf, the P contents of the sheaths were lower than their corresponding laminae. The data relating to the P content of the partitioned third leaf showed similar trends to those identified in Trial 1 (Table 5.1). The lamina (L3La) had a P value significantly higher ($P<0.05$) than that of the midrib (L3M) and lower section of the lamina (L3R), but similar to (not significantly different from) that of the top section of the lamina. The stalk was found to have the highest P content, which was significantly different ($P<0.01$) from all the other plant parts except the spindle.

Table 5.2. Effects of moisture stress, sampling date and plant parts on plant P content.

Moisture stress	Plant P (%)	Sampling date (days after planting)	Plant P (%)	Plant parts (spindle, leaf and sheath numbers)	Plant P (%)
Unstressed	0.170	145	0.170	Sp[1]	0.320
Stress/relief	0.157	155	0.175	L[2]1	0.224
		165	0.128	L2	0.171
		175	0.182	L3La[3]	0.184
				L3M[4]	0.103
				L3R[5]	0.134
				L3T[6]	0.164
				L4	0.151
				L5	0.118
				L6	0.095
				S[7]2	0.249
				S3	0.146
				S4	0.082
				S5	0.072
				S6	0.059
				St[8]	0.341
SE	0.003		0.005		0.009
LSD (0.05)	0.009		0.012		0.025
LSD (0.01)	0.012		0.016		0.031

[1]Sp = spindle; [2]L = Leaf; [3]La = lamina (mid 200mm section with midrib removed); [4]M = midrib (from 200mm section); [5]R = lower section of leaf (between the sheath and the 200mm section); [6]T = top section of the leaf (between the 200mm section and the tip); [7]S = sheath; [8]St = stalk

In considering the whole plant, the analysis of variance indicated that the moisture stress x sampling date interaction was significant at the P<0.01level (Figure 5.4). The significant difference in total plant P (%) that was apparent between the unstressed and stress/relief treatments on the first sampling date (145 days after planting) had increased substantially by the second sampling date (155 days after planting and 15 days after water was withheld). However, once stress was relieved the total P (%) of the cane associated with the stress/relief treatment increased substantially and was very similar to that of the unstressed treatment by day 175 after planting. The unexpected decline in the total P (%) in the unstressed cane on day 165 after planting, was further evidence of unrepresentative conditions associated with this treatment on that sampling date.

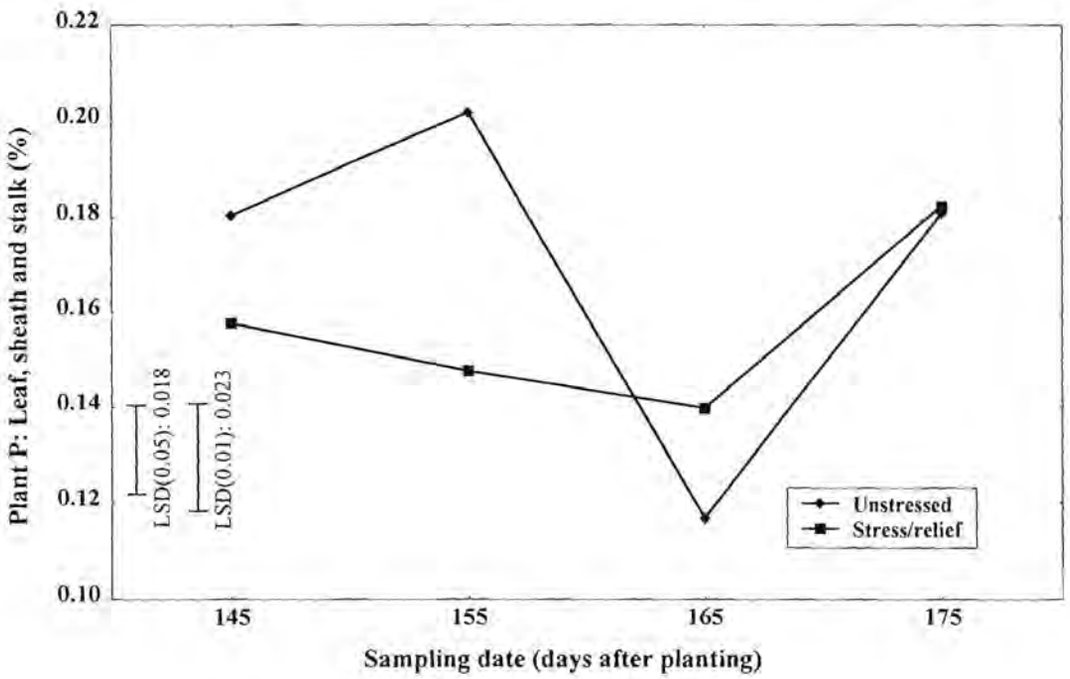


Figure 5.4. The interactive effect of moisture stress and sampling date on total plant P.

Although the moisture stress x plant part interaction was not significant, the plant P (%) values of different plant parts associated with the two stress treatments are shown (Figure 5.5) to illustrate the similarity of data, with significant differences only being apparent in the older laminae and the sheath of the second leaf.

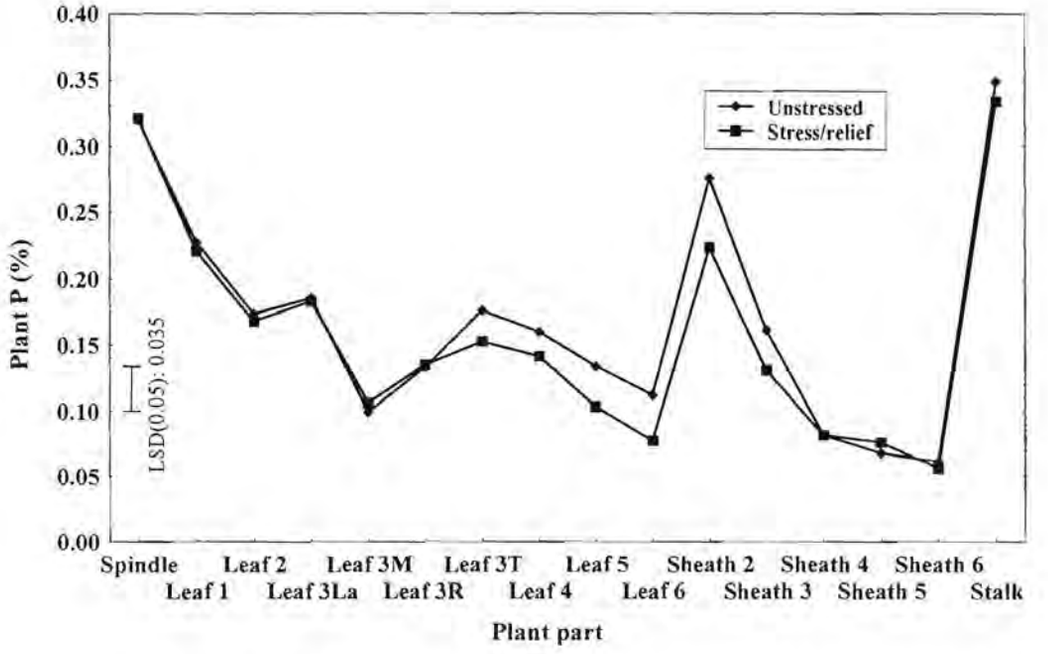
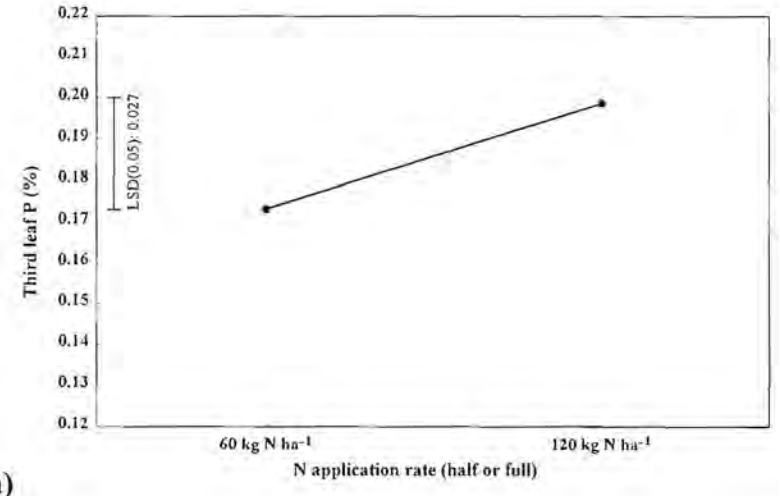


Figure 5.5. Plant P (%) of the different plant parts associated with the two moisture stress treatments.

When only the third leaf values were considered, it was found that differences in the third leaf P content (%) associated with the two N application rates (60 and 120 kg N ha^{-1}) were not significantly different from each other (Figure 5.6(a)). Likewise, there was no significant difference between the third leaf P values associated with the unstressed and stress/relief treatments (Figure 5.6(b)). The third leaf P (%) values associated with the first (145 days after planting), second (155 days after planting) and fourth (175 days after planting) sampling were not significantly different from each other (Figure 5.6(c)).

When the various interactions were considered, it was found that in terms of third leaf P (%), there was a significant interaction ($P<0.05$) between N applied and moisture stress (Figure 5.7). With unstressed conditions, the third leaf P values associated with the low (60 kg N ha^{-1}) and 'normal' (120 kg N ha^{-1}) N application rates were not significantly different from each other. However with the stress/relief treatment, the third leaf P value associated with the low application rate was significantly lower than that of the 'normal' rate of N applied.



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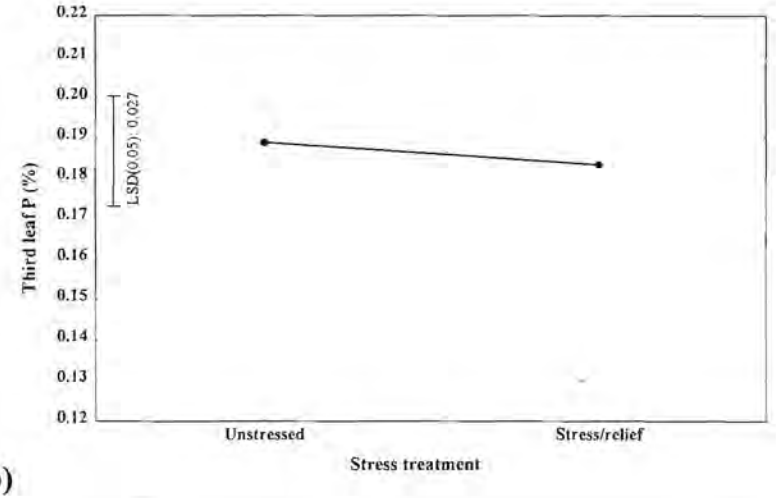

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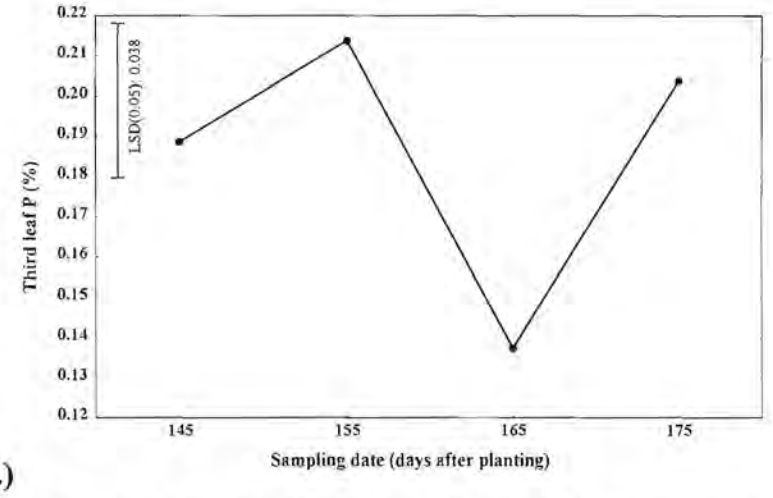


(c.)

Figure 5.6. Third leaf P (%) as affected by N application rate (a), moisture stress treatment (b) and sampling date (c).

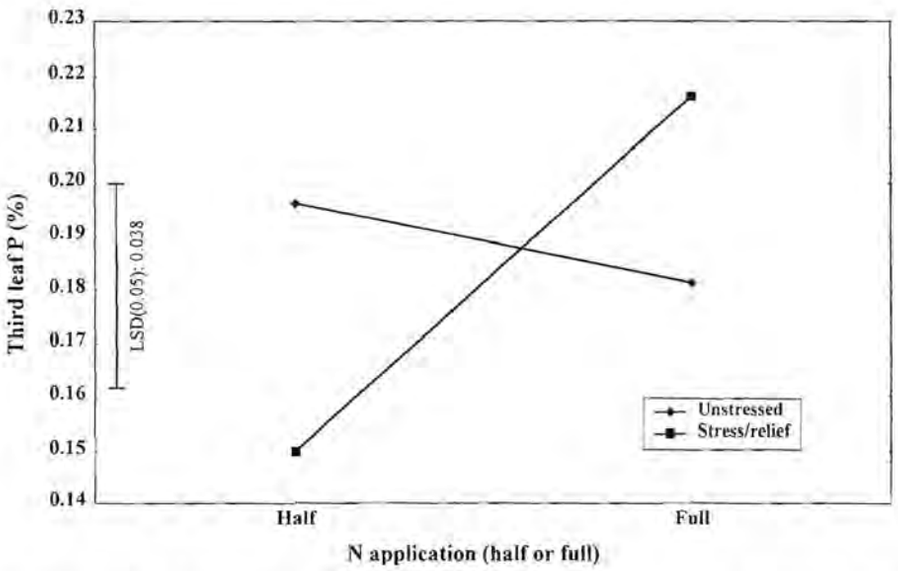


Figure 5.7. Third leaf P (%) as affected by the interaction between N applied and moisture stress treatment.

5.3.3 Effect of moisture stress on plant K (Trial 1)

The main effects associated with the analysis of variance (four moisture stress treatments, four sampling dates and 15 plant parts) indicated that moisture stress treatments (unstressed, stressed (early), stressed (late) and stress/relief) resulted in highly significant differences in mean plant K (%) values (Table 5.3), as did the partitioning of the plants into their various components. Sampling date had no significant effect on plant K (%). The mean plant K (%) values for the stressed (early), stressed (late) and stress/relief treatments were all significantly different from that of the unstressed cane. The plant K (%) associated with the stress/relief treatment was not significantly different from that of the stressed treatments which indicated little evidence of recovery of the K in the cane following stress relief. In relation to the various plants, it was found that although the plant K (%) values generally declined with increasing leaf and sheath number, and the K (%) content of the sheaths was significantly higher than that of the corresponding lamina. The highest K (%) value was associated with the sheath of the second leaf. The partitioned third leaf indicated that the K content of lamina decreased

from the base to the tip ie. L3R > L3La > L3T, and that the midrib had the lowest K concentration of the lamina parts.

Table 5.3. Effects of moisture stress, sampling date and plant parts on plant K content.

Moisture stress	Plant K (%)	Sampling date (days after planting)	Plant K (%)	Plant parts (spindle, leaf and sheath numbers)	Plant K (%)
Unstressed	2.86	100	2.59	Sp[1]	2.57
Stressed (early)	2.65	110	2.68	L[2]1	2.82
Stressed (late)	2.56	120	2.81	L2	2.49
Stress/relief	2.60	130	2.60	L3La[3]	1.75
				L3M[4]	2.73
				L3R[5]	3.17
				L3T[6]	1.47
				L4	1.93
				L5	1.61
				L6	1.17
				S[7]2	4.63
				S3	4.13
				S4	3.55
				S5	3.32
				S6	2.68
SE	0.02		0.02		0.13
LSD (0.05)	0.18		0.18		0.35
LSD (0.01)	0.24		0.24		0.46

[1]Sp = spindle; [2]L = Leaf; [3]La = lamina (mid 200mm section with midrib removed); [4]M = midrib (from 200mm section); [5]R = lower section of leaf (between the sheath and the 200mm section); [6]T = top section of the leaf (between the 200mm section and the tip); [7]S = sheath

In further consideration of the whole plant, the analysis of variance indicated a significant interaction between moisture stress x sampling date (Figure 5.8). Although the plant K (%) values associated with the stressed (early) were generally lower than those of the unstressed treatment, the differences were either not significant or only marginally significant. The same trend was generally apparent when the K (%) values of stressed (late) treatment were compared to those of the unstressed cane. Initially (first sampling: 100 days after planting and ten days after water was withheld), the K (%) value associated with the

stress/relief treatment was significantly lower than that of the unstressed cane, but not significantly different from the values associated with the other stressed treatments. However, with stress relief the K (%) values increased with time and by the fourth sampling (day 130 after planting and 20 days after re-watering), there was no significant difference between the K (%) associated with the unstressed and stress/relief treatments.

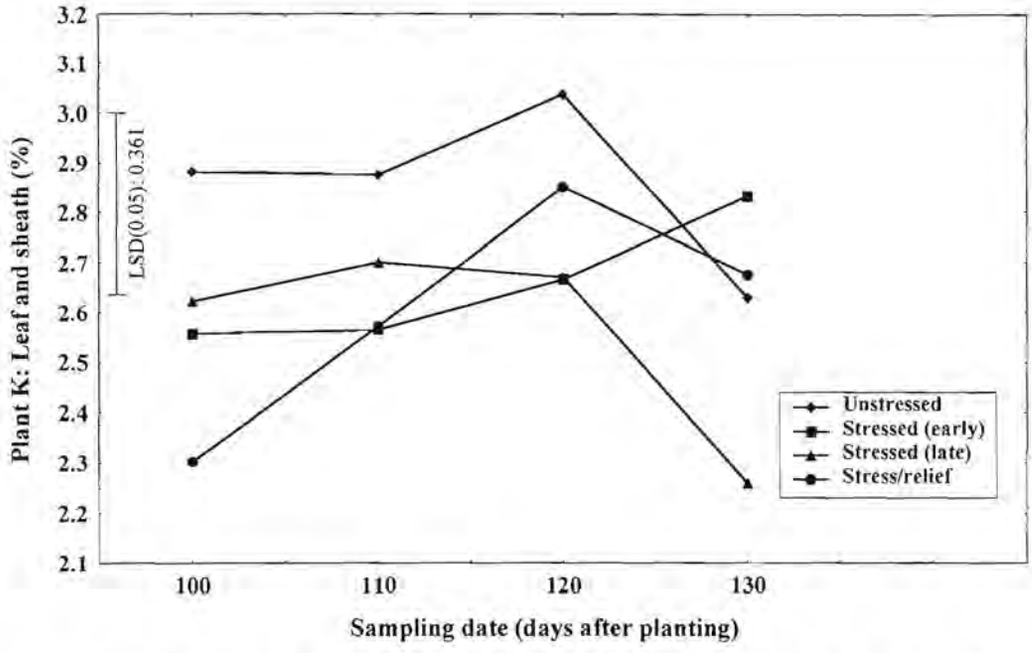


Figure 5.8. The interactive effect of moisture stress treatment and sampling date on total plant K (%).

Although the interaction between moisture stress treatment and the different plant parts did not indicate significant differences in plant K (%) content, the interactive plot is shown here for illustrative purposes (Figure 5.9). Generally the plant K values of the various plant parts were very similar to each other irrespective of moisture stress, with the only evidence of significant differences being associated with the lower portion of the lamina of the third leaf and the second leaf sheath.

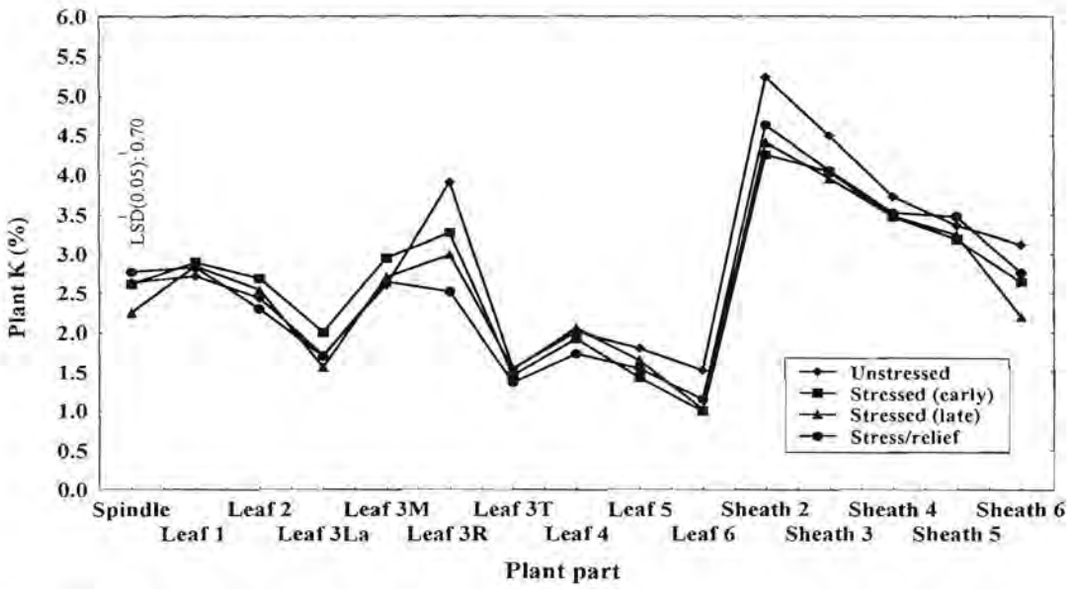
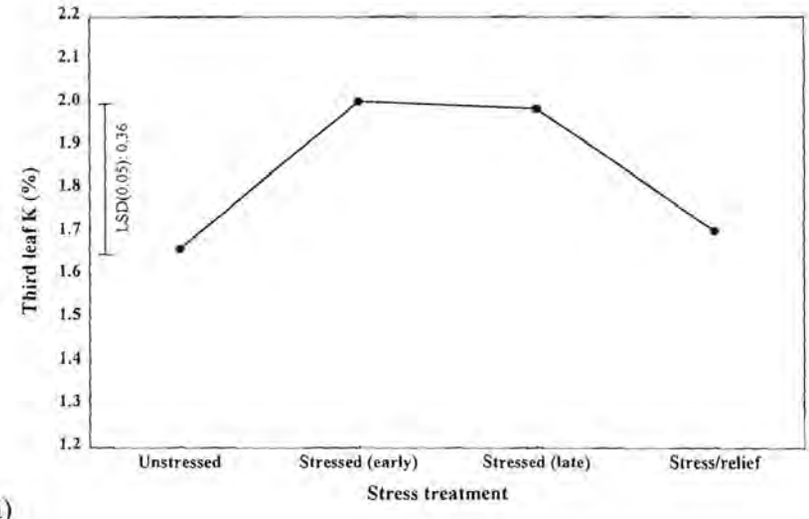


Figure 5.9. Plant K (%) of the different plant parts associated with the four moisture stress treatments.

In considering the third leaf K data only, the analysis of variance indicated no significant main effects or interactions (Figure 5.10). As such, third leaf K values were unaffected by moisture stress or sampling date.



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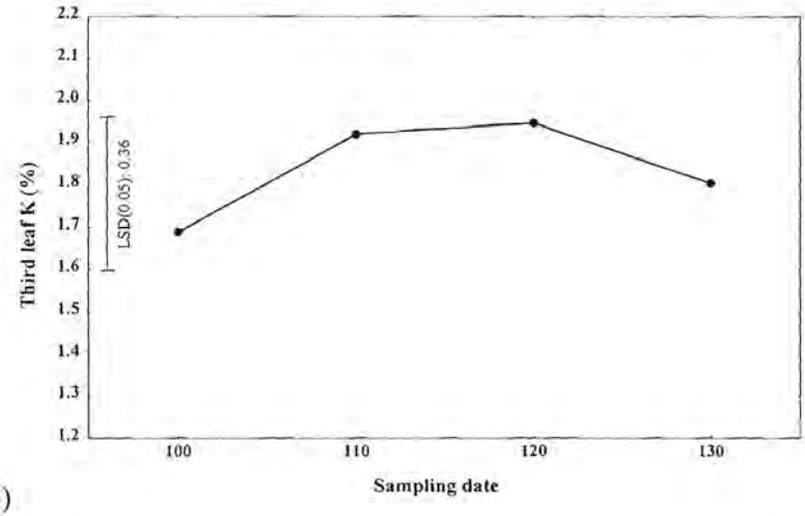

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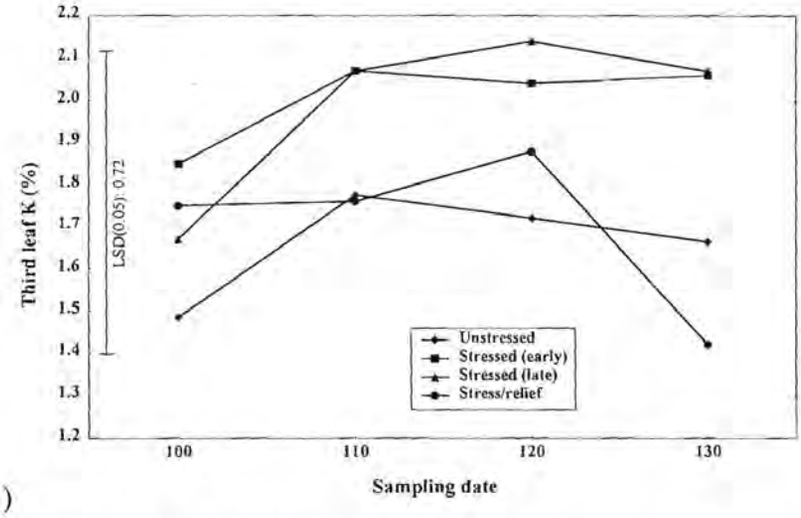


(c.)

Figure 5.10. Third leaf K (%) as influenced by moisture stress treatment (a), sampling date (b) and the interaction between moisture stress and sampling date (c).

5.3.4 Effect of moisture stress on plant K (Trial 2)

The analysis of variance (two moisture stress treatments, four sampling dates and 16 plant parts) showed that the moisture stress treatments did not result in significant differences between the total plant K (%) associated with unstressed cane and that of the stress/relief treatment (Table 5.4). However, significant differences in plant K (%) resulted from sampling date and the partitioning of the plants into their various parts. Plant K (%) declined significantly with harvest date. In relation to the partitioned plants, it was found that as in Trial 1, plant K (%) declined with increasing leaf and sheath number. As before, the various sheathes had K (%) contents significantly higher than those of the associated laminae. The sheath of the second leaf again had the highest K (%) value. In this case however, the stalk that had formed also had a substantial K concentration (3.45%). In the case of the partitioned third leaf, it was again found that the K content of the lamina decreased from base to tip.

Further assessment of the whole plant indicated that the moisture stress x sampling date was the only interaction that reached significance (Figure 5.11). Although the total plant K (%) values associated with the two moisture stress treatments were initially similar (at 145 days after planting), a separation of values occurred as the moisture stress effects became more manifest within the stress relief treatment. However with re-watering, the total K (%) values associated with the stress/relief treatment again appeared to improve to a mean value similar to the unstressed treatment. As in the case of plant P, the very low K value associated with the third sampling date cannot be explained.

Table 5.4. Effects of moisture stress, sampling date and plant parts on plant K content.

Moisture stress	Plant K (%)	Sampling date (days after planting)	Plant K (%)	Plant parts (spindle, leaf and sheath numbers)	Plant K (%)
Unstressed	2.34	145	2.56	Sp[1]	2.61
Stress/relief	2.26	155	2.41	L[2]1	2.35
		165	1.96	L2	1.88
		175	2.27	L3La[3]	1.40
				L3M[4]	2.13
				L3R[5]	2.55
				L3T[6]	1.20
				L4	1.54
				L5	1.24
				L6	1.08
				S[7]2	3.98
				S3	3.34
				S4	2.88
				S5	2.70
				S6	2.47
				St[8]	3.45
SE	0.04		0.06		0.11
LSD (0.05)	0.11		0.15		0.30
LSD (0.01)	0.14		0.20		0.40

[1]Sp = spindle; [2]L = Leaf; [3]La = lamina (mid 200mm section with midrib removed); [4]M = midrib (from 200mm section); [5]R = lower section of leaf (between the sheath and the 200mm section); [6]T = top section of the leaf (between the 200mm section and the tip); [7]S = sheath; [8]St = stalk

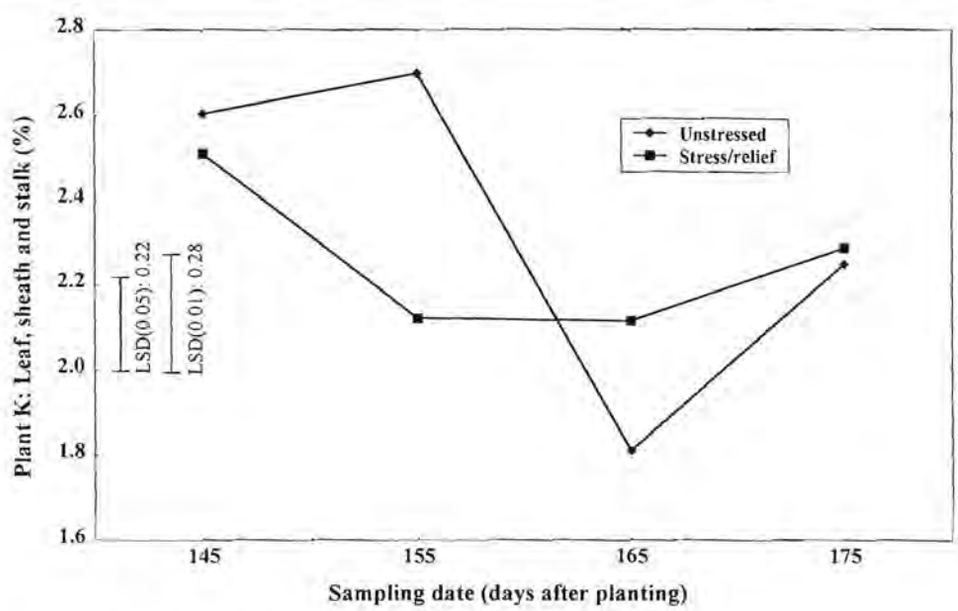


Figure 5.11. Total plant K (%) as influenced by moisture stress and sampling date.

Data relating to the effect of moisture stress on the K (%) content of the various plant components is used to illustrate the similarity of data despite the moisture stress differences (Figure 5.12). This interaction was not significant.

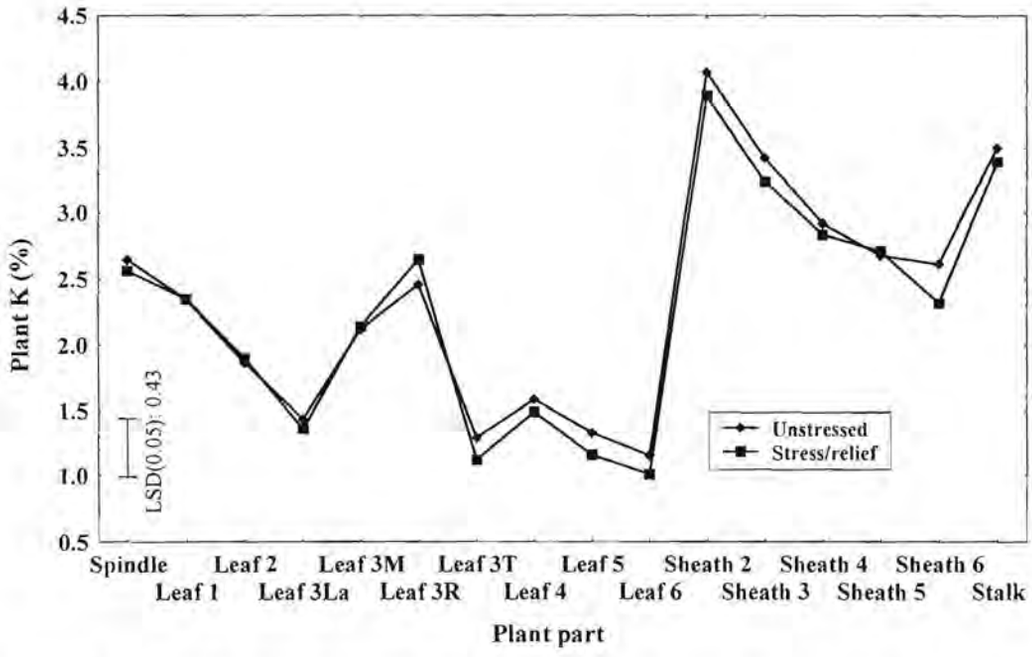
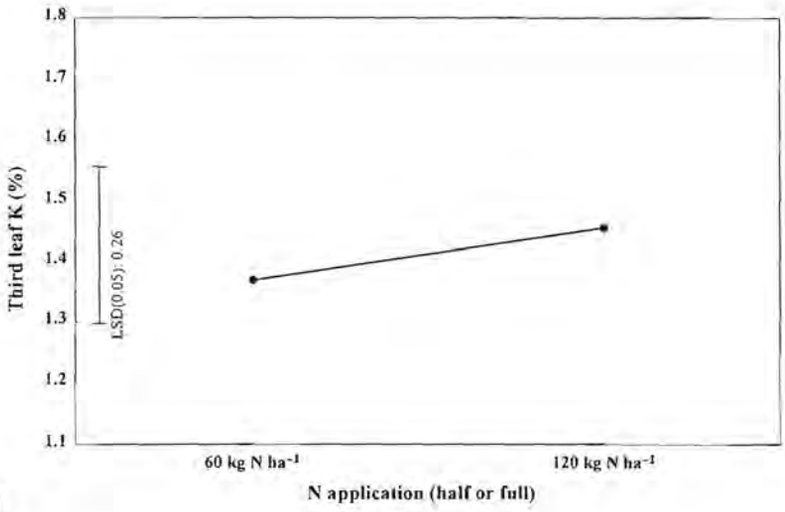
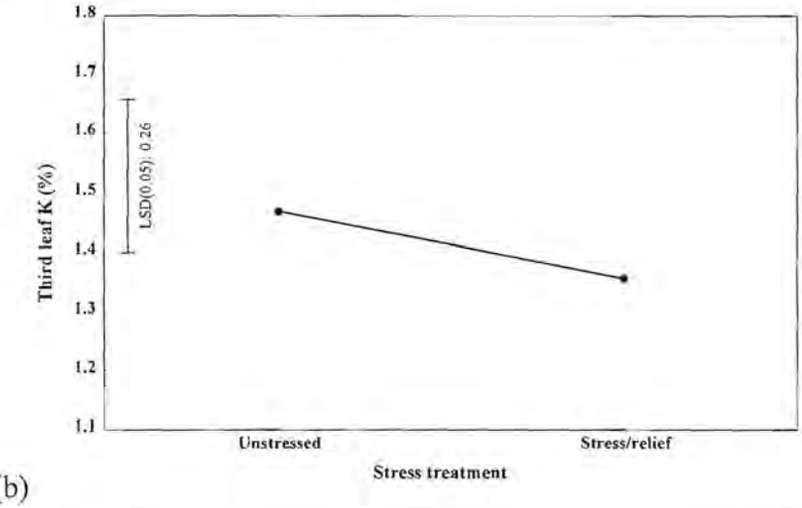


Figure 5.12. Plant K (%) of the different plant parts associated with the two moisture stress treatments.

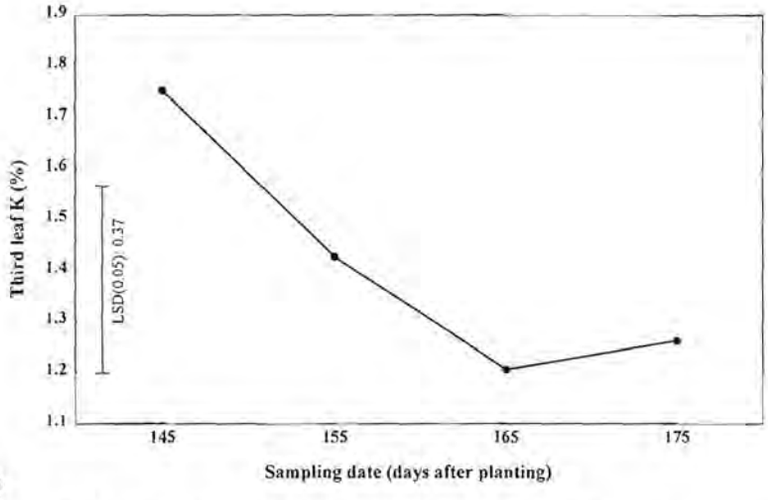
When only the third leaf data was considered, neither N application nor moisture stress treatment (unstressed and stress/relief) resulted in significant differences in leaf K content (Figure 5.13 (a) and (b)). However third leaf K (%) appeared to decline with sampling date (Figure 5.13(c). No interaction was found to be significant.



a)



(b)



(c.)

Figure 5.13. Third leaf K (%) as influenced by N application rate (a), moisture stress treatment (b) and sampling date (c).

5.4. Conclusions

The following conclusions were drawn:

- In partitioning the plants, it was found that like N, the P and K concentrations in the various plant parts differed considerably. Irrespective of the moisture status (stressed or unstressed), plant P (%) and K (%) values were found to decline with increasing leaf and sheath number. The P (%) values of the sheaths were found to be generally lower than that of the associated laminae. In contrast, the K content of the sheaths was generally higher than that of the corresponding laminae.
- In relation to the partitioning of the third leaf, it was evident that significant differences in N (Chapters 3 and 4), P and K concentrations existed in the various components (top, middle and base sections of the lamina, and midrib). This confirmed the necessity to adhere to strict sampling procedures to enable meaningful comparison of leaf nutrient values with established critical values.
- It was generally found that total plant P and third leaf P were less sensitive than plant N to moisture stress effects. Although leaf P did decline with increasing moisture stress, the effect did not appear to be as marked as that noted with leaf N. When stress was relieved, this trend was reversed with the P (%) content generally increasing throughout the whole plant.
- No strong evidence existed to suggest that critical leaf P values should reflect sampling date, as applicable for third leaf N in some of the world sugar industries.
- Plant K was generally found to be relatively insensitive to moisture stress, with little change in total plant and third leaf K with moisture stress or stress/relief.
- Unlike N and P, the K (%) content of the various plant parts remained essentially unaffected by moisture stress.
- No evidence was found to support the hypothesis that sugarcane affected by moisture stress would preferentially absorb additional K as a mechanism to increase the osmotic gradient. Any increases in the K content of sugarcane grown in drought conditions in South Africa were presumably due to soil effects ie. release of non-exchangeable K from clay minerals during wetting and drying cycles.

- As with P, no evidence was found to suggest that the third leaf critical value for K should reflect sampling date.

Chapter 6.

The interaction between the macro-nutrients (N, P and K) and moisture stress in three sugarcane varieties

6.1. Introduction

The investigation up to this point was based exclusively on sugarcane variety NCo376, the so-called 'standard variety' in the South African sugar industry. In the evaluation of sugar content, yields and agronomic traits associated with later developed South African (N) varieties, NCo376 is routinely used as a reference standard (Anon., 1995). Significant differences in varietal attributes are routinely assessed in a range of on-going variety trials throughout the industry (Anon., 1993). In relation to crop nutrition, routine leaf sampling of these and other trials has lead to recognition that differences in third leaf nutrient values exist in the various N varieties (Anon., 1995). In particular, the consistently significant differences that were observed in leaf P content between varieties NCo376 and N12, resulted in the establishment of a modified third leaf P critical value of 0.16 % for variety N12 (Schroeder *et al*, 1993). Similarly, it was found that variety N14 had third leaf K critical value 0.15 percentage units lower than the other N varieties (Donaldson *et al*, 1990). Although not 'officially' recognised within the SASEX leaf norms, there is strong evidence to suggest that the third leaf N critical value for variety NCo 310 is lower than that of NCo376 (Gosnell and Long, 1971; du Randt, 1978). On average, it would appear that this difference is about 0.1 percentage units.

Information from the SASEX variety/agronomy programme has indicated that varietal differences also exist in regard to tolerance and sensitivity to moisture stress (Anon., 1994a). In particular it was reported that variety N21 is somewhat more tolerant to moisture stress than variety NCo376 and in contrast variety N22 is more sensitive to moisture stress than NCo376.

In view of these reported differences (nutritional and reaction to moisture stress), it was considered important to explore the possible interaction between nutrients, moisture stress and some selected sugar cane varieties. As such, this investigation was aimed at assessing whether there is evidence to suggest that varietal differences existed in relation to the interaction between the macro-nutrient content of sugarcane and moisture stress conditions. As the other investigations reported in this dissertation were conducted when nutrients were adequately supplied (except in the case of Trial 2, where two rates of N were considered), the trials reported here were established under somewhat lower (yet balanced) fertility conditions. This would allow for the assessment of the interaction of moistures stress and nutrients (N, P and K) at marginal levels.

It should be noted that the investigation reported here was conducted in Brisbane, Australia, as the author had transferred from the South African Sugar Association Experiment Station at Mt Edgecombe to the Bureau of Sugar Experiment Stations (BSES) at Indooroopilly, Queensland.

6.2. Procedure

The procedure used was similar to that reported in Chapter 3, but with modifications to suite local (Australian) conditions, facilities and practices.

Two separate but concurrent trials were conducted. One (Trial 1(Qld[1]) included varieties NCo310[2] and Q141[3] and the other (Trial 2 (Qld) included varieties NCo310 and Q136. Due to strict quarantine procedures in Australia, foreign varieties cannot be easily imported into Queensland. As such, it was necessary to use locally available varieties. Due to a paucity of information relating to variety specific third leaf critical values in the Australian industry, it was considered important to utilise a South African variety as a 'semi-standard' that could be related back to NCo376 (the South African standard variety). As NCo310 had previously been widely planted in some

[1] Qld refer to Queensland, Australia
[2] Variety number beginning with N indicates that the variety was bred by SASEX
[3] variety number beginning with Q indicates that the variety was bred by BSES

areas of the Australian industry, it provided the obvious linkage. In selection of the other two varieties for the pot trial, it was decided to include Q varieties that were perceived to react differently to moisture stress. As a result, Q136 (less sensitive to moisture stress) and Q141 (more sensitive to moistures stress) (DM Hogarth – pers. comm.) were used.

The same basic procedure was used for Trial 1 (Qld) and Trial 2 (Qld):
Two sugarcane plants of uniform height (about 150mm) that had been pre-germinated from single budded setts of variety NCo310 and Q141 (Q 136) were planted into 40 litre containers (Figure 6.1) that were filled with 1600 g of dry vermiculite/perlite (1:1) mixture. This mixture is routinely used as a growth medium for propagation of sugarcane in the BSES quarantine glasshouses at Indooroopilly. Prior to planting, a commercially available compounded fertiliser and a mixture of magnesium and calcium sulphate were thoroughly mixed into the growth medium, providing a balanced and comprehensive nutrient background (Table 6.1)

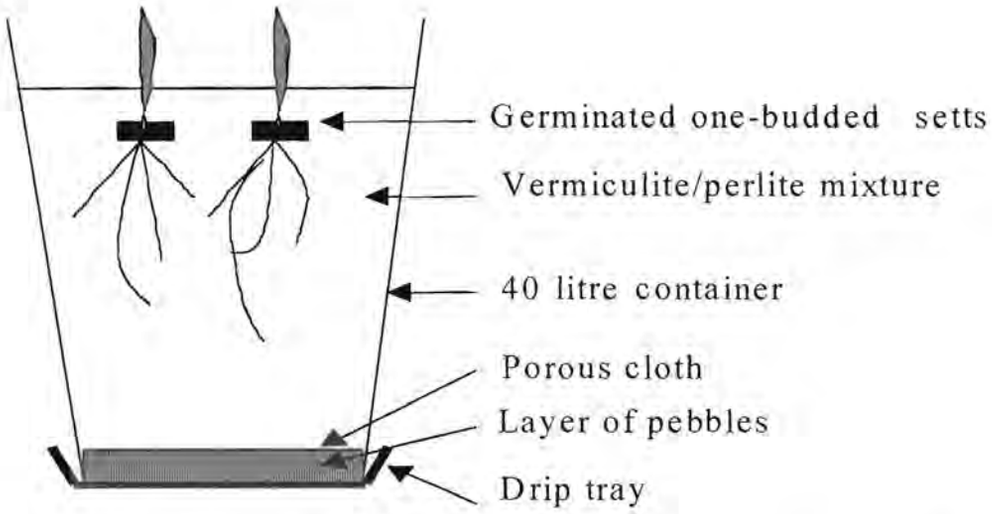


Figure 6.1 A diagrammatic representation of the planted containers used in the investigation.

Table 6.1. Nutrient quantities applied to each pot

Nutrient	Quantity of each nutrient supplied (kg/ha)
N	128
P	56
K	106
Ca	40
S	46
Mg	40
Fe	2
Mn	0.6
Cu	0.5
Zn	0.2
B	0.2
Mo	0.2

The planted containers were placed in a glass-house (fitted with evaporative coolers) on the Queensland Department of Natural Resources premises at Indooroopilly (Plate 8). They were regularly watered (every 2 to 3 days) to predetermined masses to ensure that the moisture content of the growth medium remained at approximately its water-holding capacity. Each container was positioned in a drip-tray to facilitate the return of any leachate back into the container. Once the cane had reached three months of age, moisture stress treatments were applied according to the experimental design details given below.

6.2.1. Experimental design

The experimental design was a 2 X 2 X 3 (variety X moisture stress X harvest date) factorial trial with two replications.

The moisture stress treatments were as follows:

- **Unstressed**: the growth medium was kept at its water-holding capacity until final harvest.

- **Stress/relief**: water was withheld from day 100 after planting, but stress was relieved after day 110 by watering the growth medium to its water-holding capacity once more.

The harvest (sampling) dates were as follows:

- approximately **105** days after planting
- approximately **110** days after planting
- approximately **120** days after planting

6.2.2. Experimental details

At harvest, the two plants (consisting of shoots/tillers) from each container were destructively sampled by removing all plant material to ground level. The area associated with the green leaves was measured and the LAI of the plants from each container was estimated from the sum of the total green leaf area. LAI values were expressed as area of green leaf (m^2) per surface area of soil in each container (m^2). The harvested plants were then partitioned and composite samples were formed according to leaf and sheath number, trash and stalk, if present. These composite component samples were weighed, dried in a forced draught oven at 70°C and re-weighed. Total dry matter yield was calculated by summing the individual masses and expressed as t ha^{-1}. The leaf tissue of the middle 200mm section of the lamina (L3L) was finely ground and passed through a 0.5mm perforated screen and then chemically analysed (for N, P and K) according to standard procedures in the BSES laboratory (Appendix A).

The moisture content of the growth medium was calculated for each pot at harvest according to the equation:

Growth medium moisture content (%) = $((m_f - (m_c + m_s + m_{wp})) \times 100)/m_s$

where:

mf is the final mass of the container plus total contents
m_c is the original mass of the container (including additionals)
m_s is the original mass of the dry growth medium added to the container
m_{wp} is the total wet mass of the harvested plants.

6.3. Results and discussion

6.3.1. Effect of moisture stress on LAI and dry matter production in relation to sugarcane varieties NCo310 and Q141 (Trial 1(Qld))

Although the vermiculite/perlite growth medium had a relatively high water holding capacity (moisture content of about 150 to 180% when it was allowed to freely drain), withholding water depleted this supply of moisture quite rapidly. In Trial 1 (Qld) the moisture content associated with the stress/relief treatment fell from about 150% to less than 25% (Figure 6.2) in the five day period after water was withheld (between days 100 and 105 after planting). The 'drought conditions' associated with the stress/relief treatment were maintained until rewatering from day 110 after planting (Figure 6.2). In comparison, the moisture content of the growth medium in the unstressed cane remained high (above 150%) during the full 20-day sampling period.

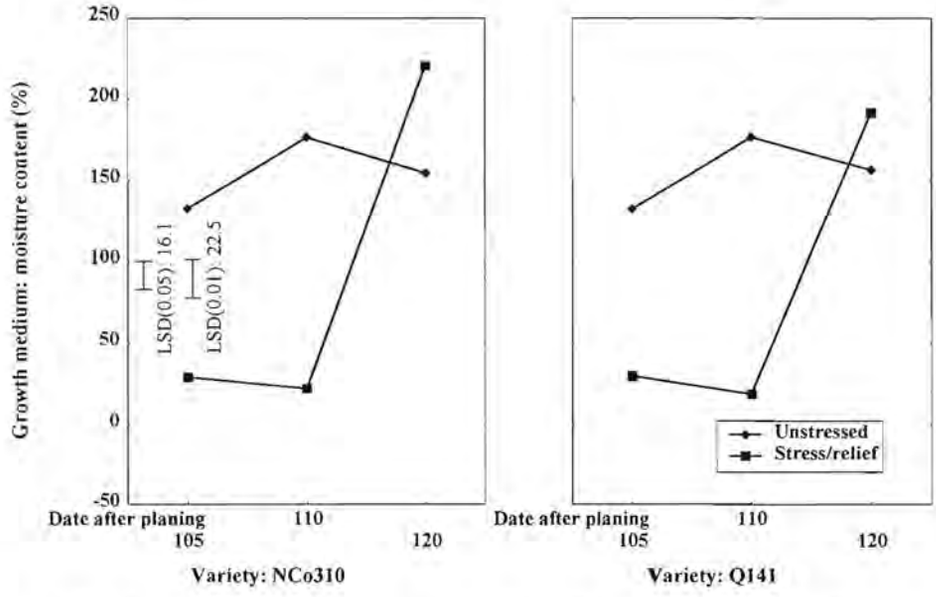


Figure 6.2. Mean moisture content values (%) of the vermiculite/perlite growth medium associated with the unstressed and stress/relief treatments for the two varieties (NCo310 and Q141) during the 20-day sampling period.

As expected, the moisture stress treatments (unstressed and stress/relief) influenced both LAI and dry matter production.

When considering the main effects - variety, moisture stress and sampling date (Table 6.1), it was found that the differences in LAI between the two varieties (NCo310 and Q141) and those related to the various sampling dates (105, 110 and 120 days after planting) were not significant. However as expected, the moisture stress treatments (unstressed and stress relief) resulted in highly significant differences ($P<0.01$) in LAI. In addition there was also a significant interactive effect ($P<0.05$) of moisture stress treatment and date of sampling on LAI (Figure 6.3). While the mean LAI values associated with the unstressed conditions increased with sampling date, the mean LAI values associated with the stress/relief treatment decreased significantly with sampling date during the "drought" conditions, with evidence of some recovery after rewatering (day 110 after planting). The differences in LAI associated with the two moisture stress treatments (unstressed and stress/relief), which were already significant on day 105 after planting (five days after water was withheld), widened further with increased moisture stress (as indicated for day 110 after planting).

Table 6.1. Effects of variety, moisture stress and date of sampling on LAI.

Variety	LAI	Moisture stress treatment	LAI	Sampling date (days after planting)	LAI
NCo310	3.303	Unstressed	4.771	105	3.683
Q141	3.711	Stress/relief	2.243	110	3.201
				120	3.638
SE	0.162		0.162		0.197
LSD (0.05)	0.527		0.527		0.645
LSD (0.01)	0.766		0.766		0.938

The three-way interaction (variety x moisture stress treatment x sampling date), although not significant, is shown in Figure 6.4 to illustrate that similarity in mean LAI values associated with the two varieties (NCo310 and Q141) under unstressed and stress/relief conditions.

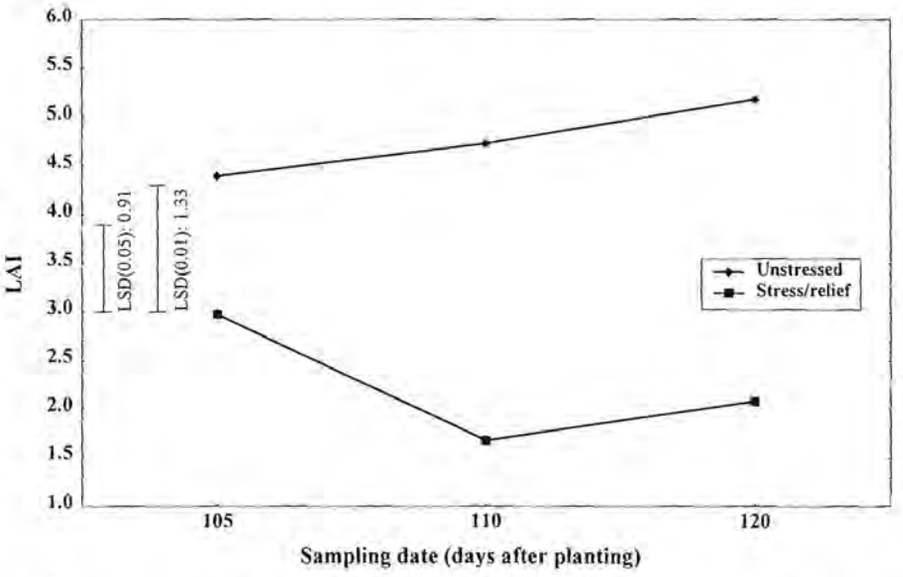


Figure 6.3. Interactive effect of moisture stress treatment (unstressed and stress/relief) and sampling date (days after planting) on LAI.

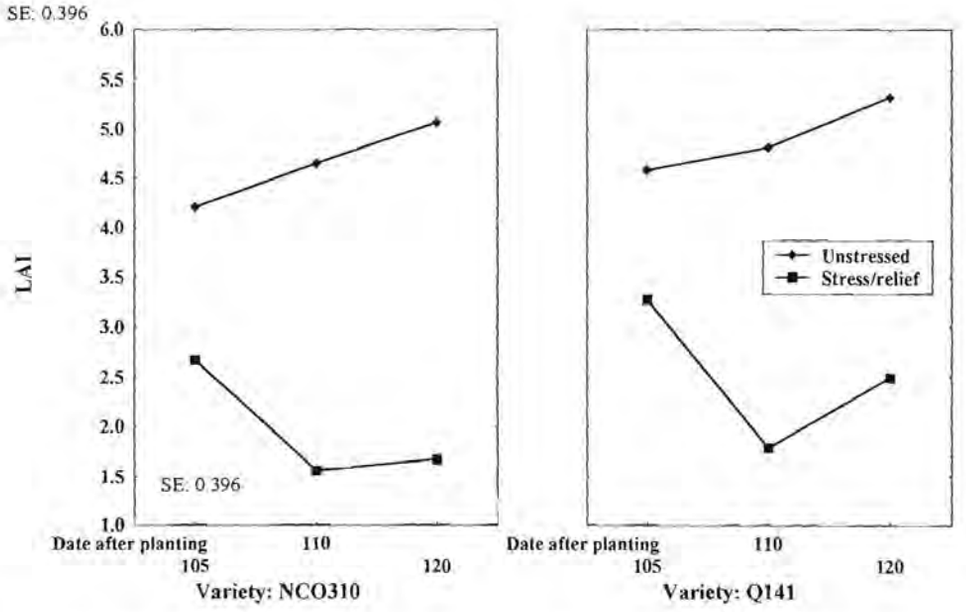


Figure 6.4. Similarity in LAI values associated with varieties NCo310 and Q141 under unstressed and stress/relief conditions.

When the main effects associated with biomass accumulation were considered it was found that, as with LAI, no varietal differences were apparent. However, both

moisture stress treatment (unstressed and stress/relief) and sampling date (days after planting) resulted in significant differences in dry matter production (Table 6.2). In particular, the dry matter yield associated with the stress/relief treatment was significantly lower ($P<0.01$) than that of the unstressed cane.

Table 6.2. Effects of variety, moisture stress and date of sampling on dry matter production.

Variety	Dry matter ($t\ ha^{-1}$)	Moisture stress treatment	Dry matter ($t\ ha^{-1}$)	Sampling date (days after planting)	Dry matter ($t\ ha^{-1}$)
NCo310	13.44	Unstressed	14.43	105	12.03
Q141	12.35	Stress/relief	11.35	110	12.62
				120	14.03
SE	0.37		0.37		0.45
LSD (0.05)	1.14		1.14		1.39
LSD (0.01)	1.59		1.59		1.95

Although no significant interactive effects were indicated in relation to biomass yield, the plot of dry matter production against sampling date for the two moisture stress treatments is shown in Figure 6.5. It illustrates the trend in biomass accumulation with time associated with unstressed conditions versus the limited growth that resulted from the imposition of moisture stress.

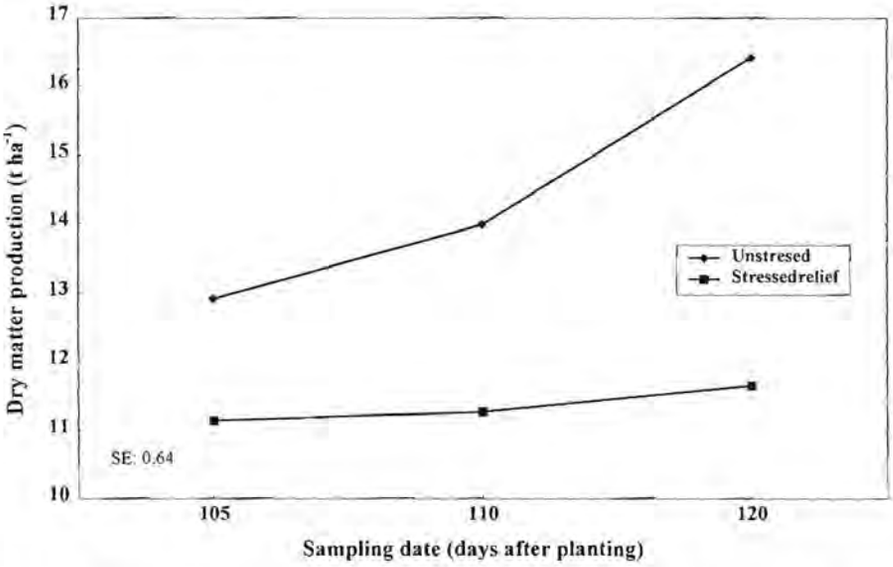


Figure 6.5. Dry matter production as affected by moisture stress and sampling date.

6.3.2. Interaction between moisture stress and plant nutrients (N, P and K) in sugarcane varieties NCo310 and Q141.

In assessing the nutrient levels of the sugarcane plants in this trial, only the third leaf nutrient values were considered as these were confirmed to be reliable indices of the overall nutrient status of a crop under both moisture stressed and unstressed conditions (Chapters 4 and % of this dissertation).

Third leaf N (%)

The main effects (variety, moisture stress treatment and sampling date) associated with the analysis of variance indicated that although date of sampling had a highly significant effect ($P<0.01$) on the third leaf N (%) values, neither variety (NCo310 and Q141) nor moisture stress treatment (unstressed and stress/relief) resulted in values significantly different from one another (Table 6.3).

Table 6.3. Effects of variety, moisture stress and date of sampling on third leaf N (%).

Variety	Third leaf N (%)	Moisture stress treatment	Third leaf N (%)	Sampling date (days after planting)	Third leaf N (%)
NCo310	1.314	Unstressed	1.259	105	1.230
Q141	1.263	Stress/relief	1.318	110	1.196
				120	1.440
SE	0.029		0.029		0.036
LSD (0.05)	0.096		0.096		0.118
LSD (0.01)	0.140		0.140		0.171

However, the highly significant ($P<0.01$) interactive effect between moisture stress treatment (unstressed and stress/relief) and date of sampling (Figure 6.6) indicated that substantial improvement in the mean third leaf N (%) value once moisture stress had been relieved (within the stress/relief treatment). None of the other interactions were found to be significant.

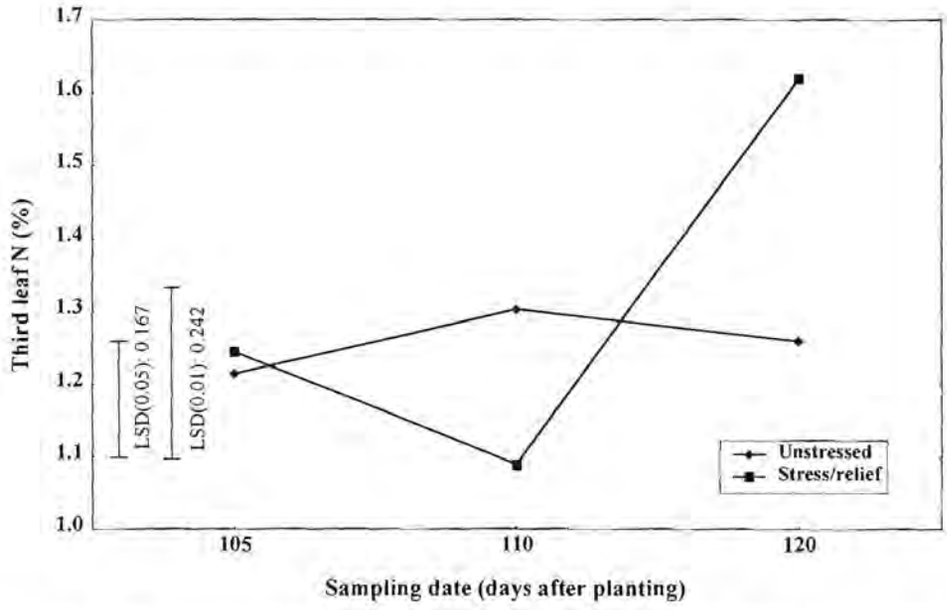


Figure 6.6. The interactive effect of moisture stress and sampling date on third leaf N (%) values.

Third leaf P (%)

The analysis of variance relating to the third leaf P (%) values showed significant main (Table 6.4) or interactive effects. The plot of third leaf P (%) against date of sampling for the two moisture stress treatments is shown in Figure 6.7 to illustrate the similarity between the leaf P values associated with the moisture stress treatments on the various sampling dates.

Table 6.4. Effects of variety, moisture stress and date of sampling on third leaf P (%).

Variety	Third leaf P (%)	Moisture stress treatment	Third leaf P (%)	Sampling date (days after planting)	Third leaf P (%)
NCo310	0.131	Unstressed	0.111	105	0.119
Q141	0.121	Stress/relief	0.141	110	0.123
				120	0.136
SE	0.013		0.013		0.016
LSD (0.05)	0.043		0.043		0.053

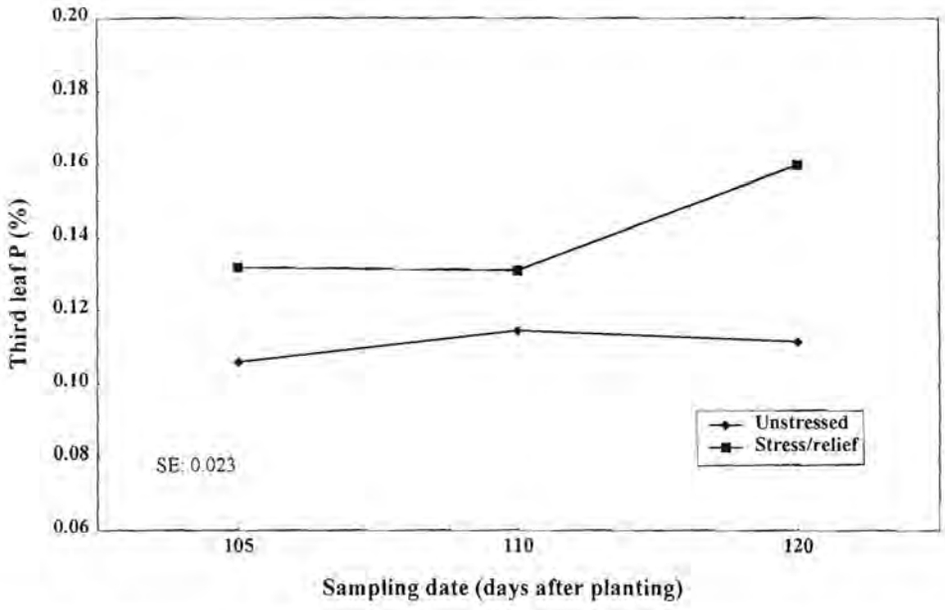


Figure 6.7. Third leaf P (%) values associated with the unstressed and stress/relief treatments on the various sampling dates.

Third leaf K (%)

As in the case of the third leaf P values, no significant differences were found to be associated with neither the main – variety, moisture stress treatments and sampling date (Table 6.5), nor interactive effects. The interaction between moisture stress treatment and sampling date (Figure 6.8) illustrates the similarity in leaf K values associated with the unstressed and stress/relief treatments, particularly after re-watering.

Table 6.5. Effects of variety, moisture stress and date of sampling on third leaf K (%).

Variety	Third leaf K (%)	Moisture stress treatment	Third leaf K (%)	Sampling date (days after planting)	Third leaf K (%)
NCo310	1.056	Unstressed	1.026	105	1.061
Q141	1.037	Stress/relief	1.067	110	1.053
				120	1.026
SE	0.016		0.016		0.019
LSD (0.05)	0.051		0.051		0.063

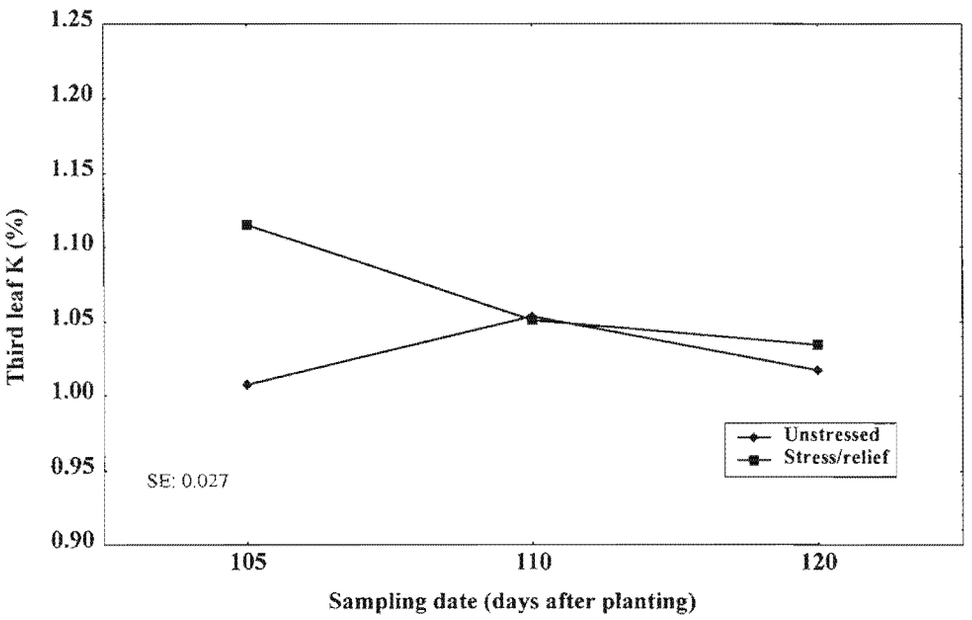


Figure 6.8. Third leaf K (%) values associated with the unstressed and stress/relief treatments on the various sampling dates.

6.3.3. Effect of moisture stress on LAI and dry matter production in relation to sugarcane varieties NCo310 and Q136 (Trial 2(Qld))

As in the case of Trial 1 (Qld), withholding water (within the stress/relief treatment) resulted in the moisture content of the vermiculite/perlite growth medium being substantially depleted (Figure 6.9) and giving rise to conditions that would simulate "drought" effects. In the case of the unstressed treatment, the moisture content of the growth medium was kept above 150%. That the moisture content of the growth medium at 'field capacity' in Trial 2 (Qld) appeared to be slightly higher than that noted in Trial 1 (Qld) (Figure 6.2) was thought to be related to small differences in the ratio of vermiculite to perlite. However, due to the relatively large water holding capacity of the growth medium these differences were not considered important. In relation to the stress/relief treatment, rewatering on day 110 after planting increased the moisture content of the growth medium from about 18% to approximately 180%. The analysis of

variance indicated that there were no significant differences in the moisture content of the growth medium associated with the two varieties.

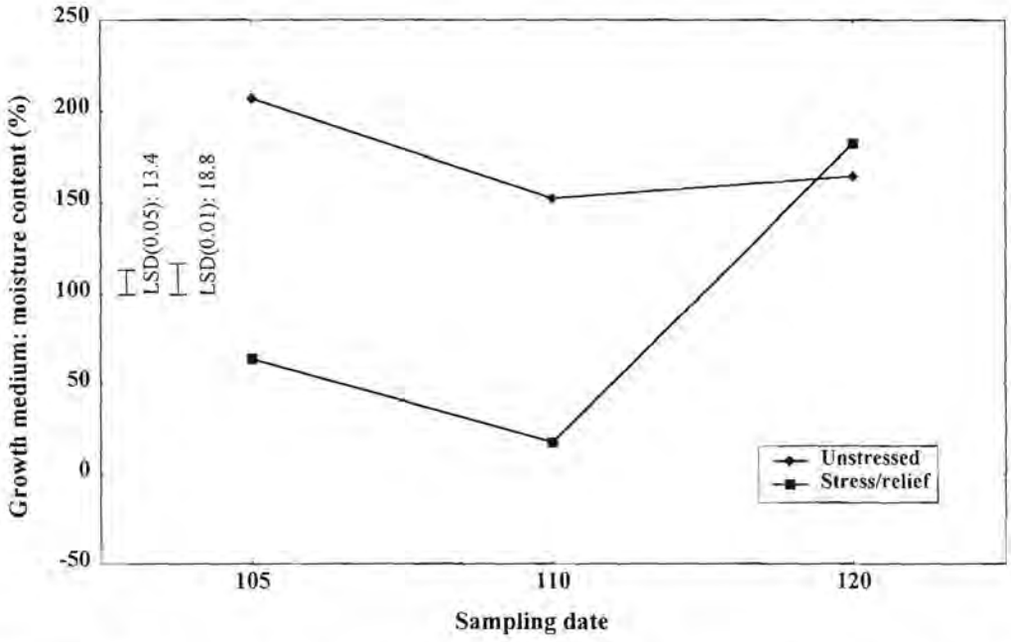


Figure 6.9. Mean moisture content values (%) of the vermicultite/perlite growth medium associated with the unstressed and stress/relief treatments during the 20-day sampling period.

As in the previous trial, the moisture stress effects, as quantified above, influenced both LAI and dry matter production.

The main effects associated with the analysis of variance of the LAI data indicated that while the moisture stress treatments and sampling date had highly significant effects ($P<0.01$) on LAI, no significant varietal differences (in relation to varieties NCo310 and Q136) were apparent (Table 6.6).

Table 6.6. Effects of variety, moisture stress and date of sampling on LAI.

Variety	LAI	Moisture stress treatment	LAI	Sampling date (days after planting)	LAI
NCo310	3.701	Unstressed	4.733	105	4.539
Q136	3.966	Stress/relief	2.934	110	2.986
				120	3.975
SE	0.087		0.087		0.107
LSD (0.05)	0.284		0.284		0.348
LSD (0.01)	0.413		0.413		0.506

The only significant interactive effect relating to LAI, was that of moisture stress x sampling date (Figure 6.10). While the mean LAI values associated with the unstressed conditions remained above 4.5 and increased slightly with time, the mean LAI value associated with the stress/relief treatment declined markedly with the imposition of moisture stress and improved once again with re-watering. Although the three-way interaction (variety x moisture stress treatment x sampling date) did not indicate significant differences, it is shown in Figure 6.11 to indicate that the two varieties (NCo310 and Q136) behaved similarly under both unstressed and stress/relief conditions.

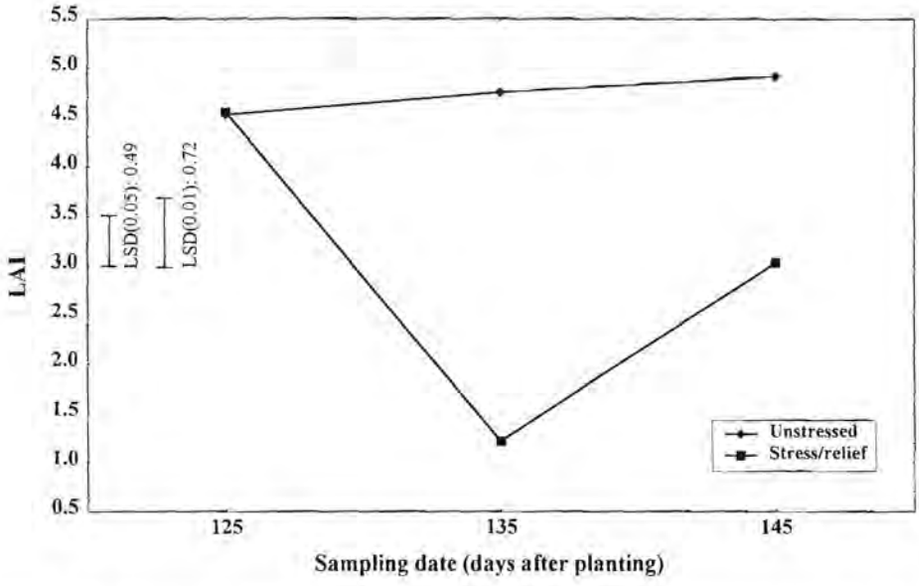


Figure 6.10. Interactive effect of moisture stress treatment (unstressed and stress/relief) and sampling date (days after planting) on LAI.

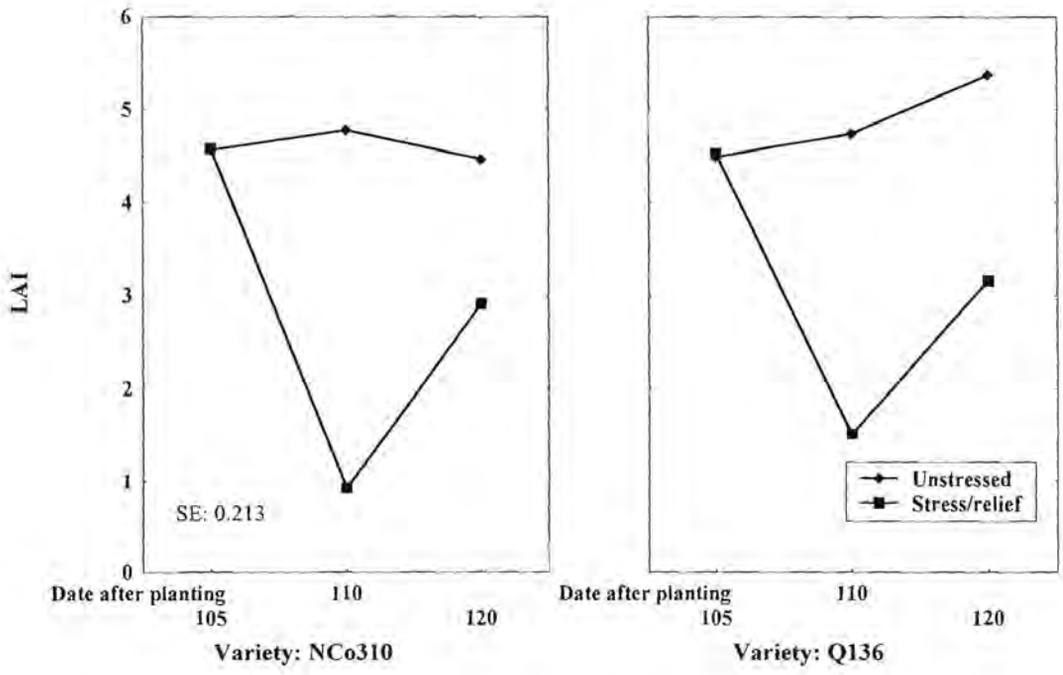


Figure 6.11. Plot of LAI versus sampling date under unstressed and stress/relief conditions for the two sugarcane varieties NCo310 and Q136.

When biomass accumulation was considered, it was found that all the main effects (variety, moisture stress treatment and sampling date) resulted in significant differences in dry matter yield values (Table 6.7). Moisture treatment x sampling date was the only significant interactive effect ($P<0.01$). Although the dry matter yields of the unstressed and stress/relief treatments were initially similar (Figure 6.12), the imposition of moisture stress severely curtailed growth (as indicated by the low mean dry matter production value associated with the stress/relief treatment for day 120 after planting). In comparison the dry matter yield associated with the unstressed conditions significantly increased with each sampling date. The dry matter yield of variety Q136 was found to be significantly lower ($P<0.01$) than that of variety NCo310. Also, but as expected, the mean dry matter yield associated with the stress/relief treatment was significantly lower ($P<0.05$) than that of the unstressed cane.

Table 6.7. Effects of variety, moisture stress and date of sampling on dry matter production.

Variety	Dry matter ($t\ ha^{-1}$)	Moisture stress treatment	Dry matter ($t\ ha^{-1}$)	Sampling date (days after planting)	Dry matter ($t\ ha^{-1}$)
NCo310	18.90	Unstressed	18.08	105	14.61
Q136	15.55	Stress/relief	16.38	110	18.02
				120	19.05
SE	0.46		0.46		0.56
LSD (0.05)	1.42		1.42		1.74
LSD (0.01)	1.99		1.99		2.43

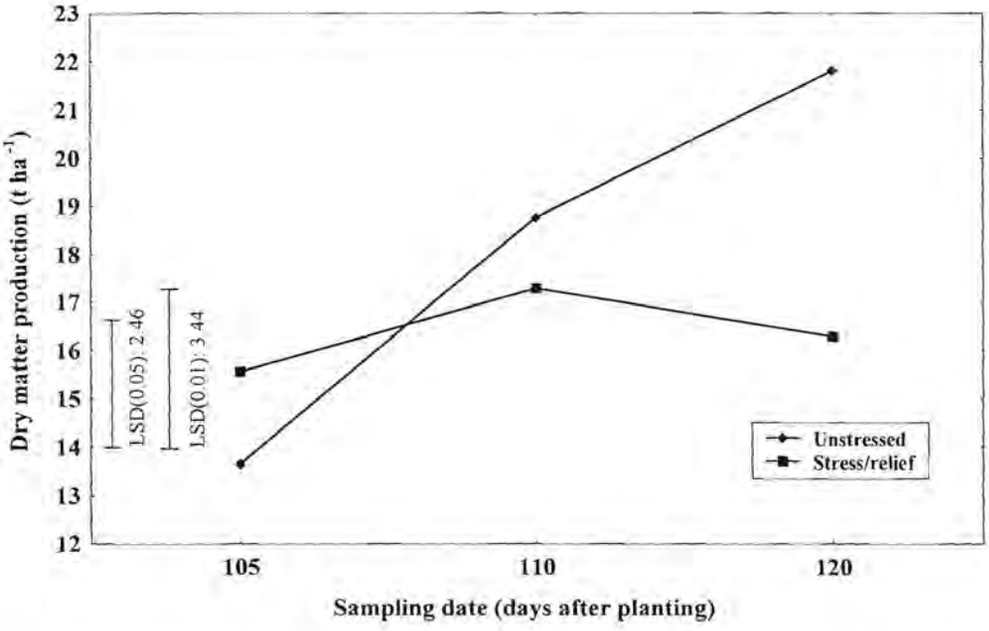


Figure 6.12. Dry matter production as affected by moisture stress and sampling date.

6.3.4. Interaction between moisture stress and plant nutrients (N, P and K) in sugarcane varieties NCo310 and Q136

As in Trial 1 (Qld) the third leaf nutrient values were used to gauge the overall nutrient status of the plants.

Third leaf N (%)

While "date of sampling" had a highly significant effect on third leaf N ($P<0.01$), no significant affects were apparent in relation to variety and moisture stress (Table 6.8).

Table 6.8. Effects of variety, moisture stress and date of sampling on third leaf N (%).

Variety	Third leaf N (%)	Moisture stress treatment	Third leaf N (%)	Sampling date (days after planting)	Third leaf N (%)
NCo310	1.285	Unstressed	1.357	105	1.426
Q136	1.328	Stress/relief	1.257	110	1.140
				120	1.354
SE	0.028		0.028		0.034
LSD (0.05)	0.090		0.090		0.110
LSD (0.01)	0.131		0.131		0.160

However, moisture stress and sampling date resulted in a highly significant interactive effect on third leaf N (Figure 6.13). Although the mean third leaf N values associated with the unstressed and stress/relief treatments were initially similar (not significantly different at 105 days after planting), the imposition of moisture stress resulted in a marked decrease in the mean third leaf N value associated with the stress/relief treatment. The third leaf N values associated with the two moisture stress treatments were significantly different from each other ($P<0.01$) on day 110 after planting. However, this difference once again returned to non-significance once the moisture stress was relieved.

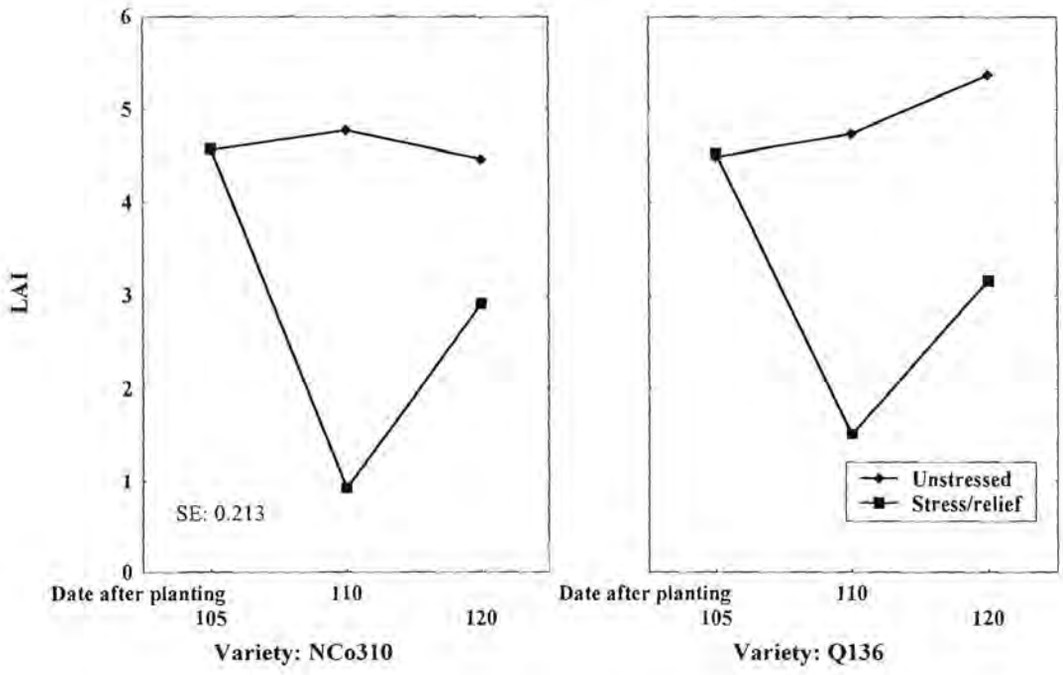


Figure 6.11. Plot of LAI versus sampling date under unstressed and stress/relief conditions for the two sugarcane varieties NCo310 and Q136.

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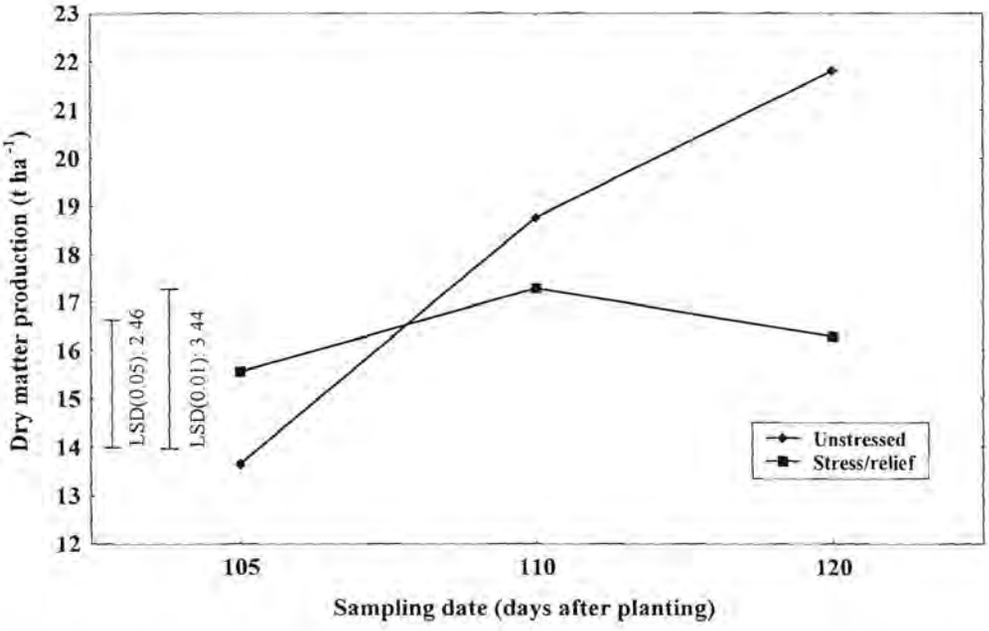

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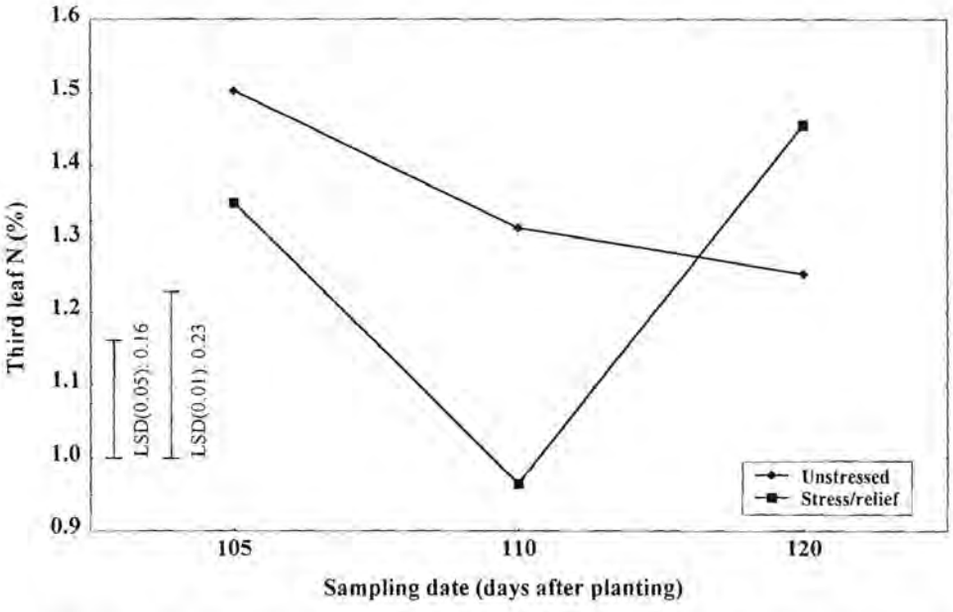

Figure 6.13. The interactive effect of moisture stress and sampling date on third leaf N values.

Third leaf P and K (%)

As in the case of Trial 1 (Qld), the analysis of variance showed no main (Tables 6.9 and 6.10) or interactive effects associated with both the third leaf P and K values. Third leaf P and K (%) values plotted against sampling date for the unstressed and stress/relief treatments (Figures 6.14 and 6.15) are included here to illustrate the similarity between values and show the non-significant differences that occurred due to moisture stress and date of sampling.

Table 6.9. Effects of variety, moisture stress and date of sampling on third leaf P (%).

Variety	Third leaf P (%)	Moisture stress treatment	Third leaf P (%)	Sampling date (days after planting)	Third leaf P (%)
NCo310	0.131	Unstressed	0.125	105	0.132
Q136	0.125	Stress/relief	0.131	110	0.126
				120	0.125
SE	0.004		0.004		0.005
LSD (0.05)	0.014		0.014		0.015

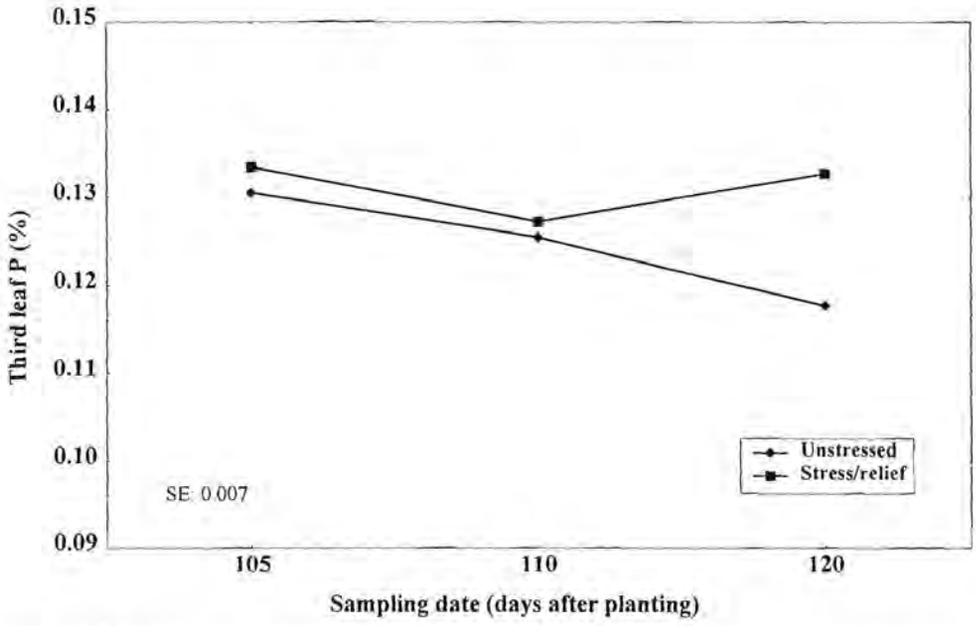

Figure 6.14. Third leaf P (%) values associated with the unstressed and stress/relief treatments on the various sampling dates.

Table 6.10. Effects of variety, moisture stress and date of sampling on third leaf K (%).

Variety	Third leaf K (%)	Moisture stress treatment	Third leaf K (%)	Sampling date (days after planting)	Third leaf K (%)
NCo310	0.997	Unstressed	1.098	105	1.139
Q136	1.195	Stress/relief	1.094	110	1.073
				120	1.076
SE	0.019		0.019		0.024
LSD (0.05)	0.063		0.063		0.077

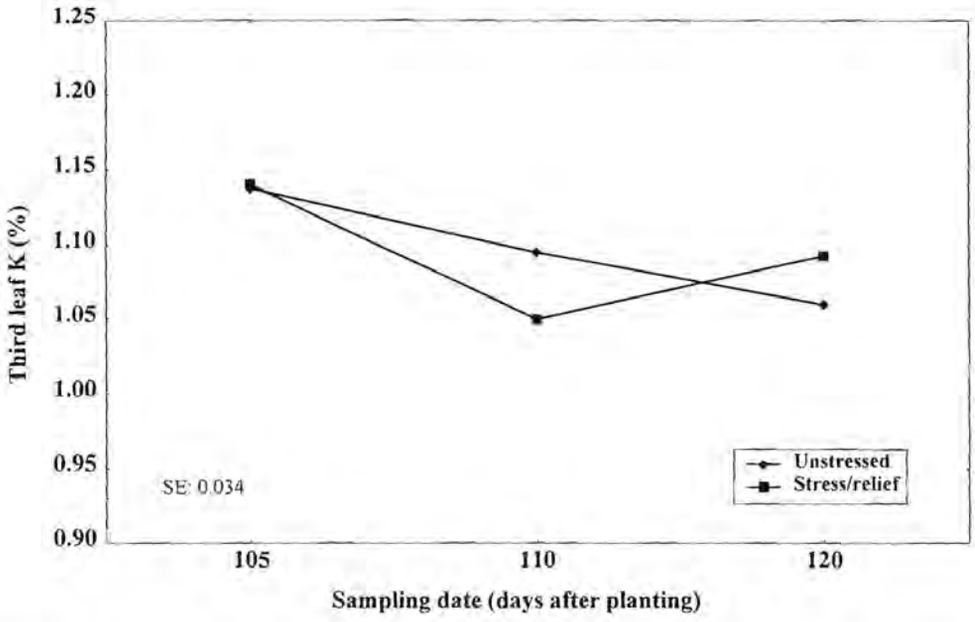

Figure 6.15. Third leaf K (%) values associated with the unstressed and stress/relief treatments on the various sampling dates.

6.4. Conclusions

The following conclusions were drawn:

- In terms of methodology, it was found that the vermiculite/perlite growth medium provided a useful and suitable way of assessing varietal differences in regard to nutrition and moisture status without introducing a 'soil' factor. This technique will be used for further studies in assessing varietal differences and nutrient interaction studies.
- The trends relating to the decline in third leaf N values under moisture stress conditions and with recovery after stress was relieved were similar to those noted in the previous investigations (Chapters 2, 3 and 4 of this dissertation). This was despite the marginal to low N values that reflected the low N application rates.
- In contrast, third leaf P values were found to be less sensitive than leaf N to moisture stress conditions with differences generally being non-significant at $P<0.05$ level. This trend was similar to that observed under high P conditions as noted in Chapter 5 of this dissertation.

- Third leaf K values appeared to be insensitive to moisture stress as the mean third leaf K values remained fairly close to the general South African third leaf critical value (Table 1.1), irrespective of the moisture stress treatment (unstressed and stress/relief) or date of sampling. The relatively low third leaf K values reflected the low K application rate.
- In terms of the third leaf nutrient values (N, P and K) there was no evidence of varietal differences under either unstressed or stress/relief conditions. As such the general critical values should remain applicable for both Q141 and Q136.

Chapter 7.

The assessment of a moisture stress indicator for improved interpretation of leaf analysis data

7.1. Introduction

Although the effect of moisture stress on third leaf nutrient (N, P and K) values is better understood due to the investigation reported in this dissertation, it was considered important to develop a robust 'moisture stress indicator' that could be used when interpreting leaf analysis data. The avoidance technique ie. sampling only when sugarcane was not affected by moisture stress (Halais, 1962) , has really been the only option to date, for ensuring meaningful interpretation of leaf analysis data. The ability to leaf sample sugarcane without this restriction would broaden and add greater flexibility to the appropriate sampling period. A moisture stress indicator used in conjunction with leaf analysis data would certainly be extremely useful in this regard.

One option that was initially considered in this investigation was a "bio-chemical" moisture stress indicator that could be determined on the plant tissue at the time of analysis. As the accumulation of amino acids in various plant parts had been investigated by scientists in various sugar industries for drought tolerance, sugar quality, fertiliser management, etc (Rutherford, 1989; Chapman *et al*, 1996; Keating *et al*, 1999), the possibility soon fell towards proline which was widely reported to accumulate under moisture stress condition in a number of agricultural crops (Rao and Asokan, 1978; Rutherford, 1989; Irrigoyen *et al*, 1992; Steyn and Rossouw, 1995). However, from literature it was established that although proline could possibly be useful in mechanisms involved with drought tolerance in sugarcane, it would not serve as a robust moisture stress indicator for use with nutrient leaf analysis data. The reasons for this included reports that proline accumulation could occur due to other plant stresses apart from moisture stress (Aspinall and Paleg,

1981), be affected by crop age, sampling season and by leaf K concentration (Rutherford, 1989), and vary according to variety (Rao and Asokan, 1978). In addition, it appeared that proline concentration in the leaf tissue was affected by conditions after sampling as shown by Rutherford (1989) in simulating 'drought conditions' using polyethylene glycol solutions. Although accumulation of proline in excised leaf could be prevented by immediately freeze-drying the leaf tissue (Anon., 1994 (b)) this facility would be unavailable to growers during routine leaf sampling.

Although the commonly used agronomic measurements, such as growth rate and LAI, certainly offer suitable tools for assessing whether moisture stress is present in glass-house experiments and field trials, they are not suitable for on-farm usage, nor can they be used for rapid assessment of moisture stress conditions.

Based on the DRIS approach (Meyer, 1981), the third leaf N:P ratio has often been used by the SASEX fertiliser advisory service as a 'rule of thumb' to assess leaf analysis data suspected of being influenced by moisture stress. In this regard, an N:P value of 10 was thought to be applicable for well nourished sugarcane (in terms of N and P) irrespective of moisture stress effects. However, this approach is problematic for at least two reasons. The first, as shown in Chapters 5 and 6 of this dissertation, is related to the fact that third leaf N and P values are not identically influenced by moisture stress effects. Differentially declining N and P values due to moisture stress would result in variable N:P values. The second concern is associated with the possibility of low third leaf P values (due to under-fertilisation) in association with moisture stress effects. A low leaf N value related to drought stress conditions in combination with a low leaf P value (due to inadequate P) could possibly result in confused interpretation of analysis data.

In light of the above, it was deemed necessary to find a moisture stress indicator that could allow easier interpretation of leaf analysis data, but would also be appropriate for general and practical use in the industry. Of particular interest was the possible use of the dry masses of the various plant components (spindle, leaf, sheath and trash)

expressed as a percentage of their wet masses (D%W). This would not only be readily available, but could easily be incorporated into leaf sampling routines.

7.2. Procedure

The data discussed here are those obtained from the four trials (Trial 1, Trial 2, Trial 1(Qld) and Trial 2(Qld)) reported in Chapters 3, 4, 5 and 6 of the dissertation. The dry masses of the various plant components (spindle, leaf, sheath and trash) expressed as a percentage of their wet masses (D%W) were calculated for all samples collected during the harvest operation in each trial. However, only those related to the spindles and top section of the third laminae (between the 200mm section used for chemical analysis and the leaf tip) were considered here. As plant N had been shown to be the nutrient most affected by moisture stress (Chapters 3, 4 and 6 of this dissertation), third leaf N values were used in the assessment of D%W as a moisture stress indicator according to the following procedure:

- A baseline of third leaf N values was established for unstressed conditions for each trial.
- Relative third leaf N values (%) were then calculated by expressing the actual third leaf (%) values associated with each sample as a percentage of the appropriate baseline value.
- D%W critical values were established by plotting the relative third leaf N values against D%W of the top section of the third leaf including the midrib (L3T) and the spindle (Sp).
- D%W (spindle) values were plotted against D%W (L3T) values to establish whether moisture stress could be predicted from the combination of these values. Data from Trial 1 and Trial 1 (Qld) were separated from the data from Trials 2 and 2(Qld) to enable a validation step.
- A regressional analysis on the data from Trial 1 and Trial 1 (Qld) was used to determine the relationship between D%W (L3T) and relative third leaf N (%). Data from Trial 2 and Trial 2 (Qld) were used for validation purposes.

7.3. Results and discussion

The baseline third leaf N values, obtained from the regression equations of mean third leaf N (L3N) values plotted against date of sampling (eg Equation 1), were established for each trial (based on unstressed conditions) and reflected the usual decline in N with age and time of sampling (Table 7.1). Two baselines were established for Trial 2 to reflect the full and half N application rates applicable in that case (Chapter 4).

$$L3N = -0.0128t + 3.80 \quad \text{..... (Equation 1)}$$

The mean relative third leaf N values (calculated by expressing the mean third leaf N (%) values as a percentage of the appropriate baseline value), as with the third leaf values, reflected the decline and subsequent increase in leaf N as stress was imposed and relieved (Table 7.1).

The full set of relative third leaf N values (from all four trials) plotted against D%W of the spindle (Figure 7.1) and third leaf (Figure 7.2) indicated that the data could be separated according to the moisture stress treatment (unstressed, stressed and stress/relief) in each trial. Generally, it was found that in the case of the spindle data, the unstressed relative third leaf N values (above 90%) could be separated from the moisture stress related data by a D%W(spindle) value of 22 (Figure 7.1). D%W (spindle) values less than 22% would indicate that the spindle was unaffected by moisture stress. In relation to the data pertaining to the top section of the third leaves, a similar separation occurred at a D%W (L3T) value of 32 (Figure 7.2). D%W (L3T) values below 32 would indicate unstressed conditions in the third leaf. However, when the data associated with stress relief were considered, it was found that although the D%W (spindle) values dropped below 22% soon after rewatering, the third leaf N values remained below a relative third leaf N value of 90% (as indicated by the closed triangles in Figure 7.1). When stress-free conditions persisted for a longer period (D%W (spindle) remaining below 22%) the relative third leaf N values increased above 90% (as indicated by the closed circles in Figure 7.1). In contrast, the D%W

(L3T) values remained above 32% (indicating that the third leaf was still affected by moisture stress) shortly after re-watering. Correspondingly, the relative third leaf N values remained below 90% (as indicated by the closed triangles in Figure 7.2). Once the D%W (L3T) values decreased to about 32% (with continuing unstressed conditions), the relative third leaf values were found to be above 90% (as indicated by the closed circles in Figure 7.2).

Table 7.1. Baseline third leaf N values related to relevant sampling dates from Trial 1 and the associated mean third leaf N values and calculated relative third leaf N values.

Trial	Sampling date (days after planting)	Calculated baseline third leaf N values (%)	Mean third leaf N values (%)				Calculated relative third leaf N values (%)			
			US[1]	SE[2]	SL[3]	SR[4]	US[1]	SE[2]	SL[3]	SR[4]
1	100	2.52	2.56	2.29	2.39	2.33	101.7	90.9	94.9	92.6
	110	2.39	2.32	1.90	2.58	1.90	97.1	79.3	108.0	79.5
	120	2.26	2.27	1.71	2.08	1.26	100.4	75.6	92.0	55.7
	130	2.13	2.15	1.57	1.87	2.25	100.8	73.6	87.7	105.5
2 (Full N)	145	2.28	2.16			2.19	94.9			96.3
	155	2.05	2.10			1.71	102.7			83.6
	165	1.82	1.78			1.04	98.1			57.3
	175	1.59	1.49			1.97	94.0			124.3
2 (Half N)	145	2.34	2.35			1.40	100.3			59.8
	155	2.00	2.12			1.17	105.9			58.5
	165	1.65	1.40			0.82	84.4			49.4
	175	1.32	1.45			1.02	110.1			77.5
1 (Qld)	100	1.40	1.41			1.24	100.7			88.6
	110	1.32	1.30			1.09	98.2			82.3
	120	1.25	1.26			1.62	100.9			129.8
2 (Qld)	100	1.48	1.50			1.35	101.2			91.1
	110	1.36	1.32			0.97	97.3			71.5
	120	1.23	1.25			1.45	101.5			117.7

Treatments: [1]US = Unstressed [2] SE = Stressed (early)
[3] SL = Stressed (late) [4] SR = Stressed/relief

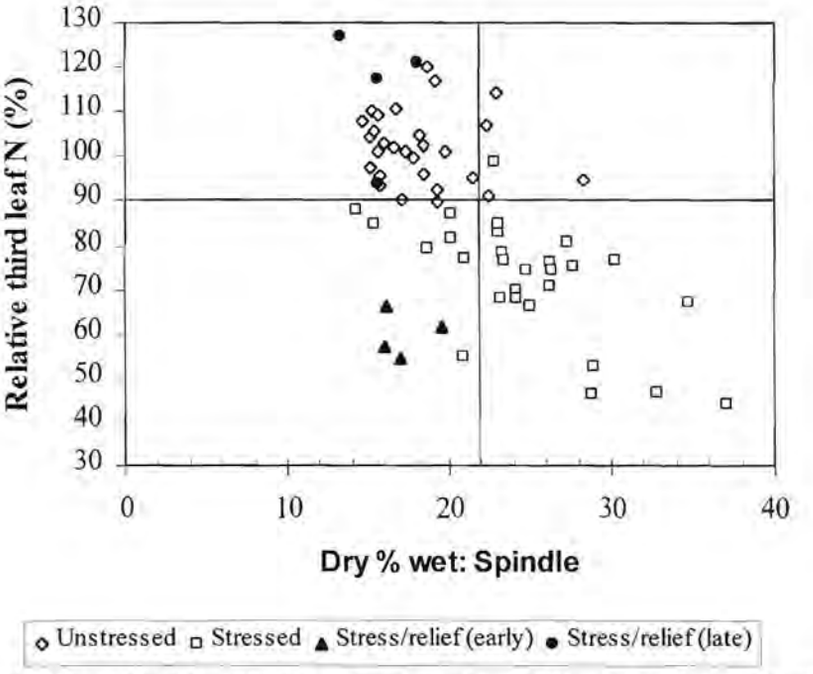

Figure 7.1. Relative third leaf N (%) plotted against D%W (spindle). D%W value of below 22% would indicate unstressed conditions in the spindle.

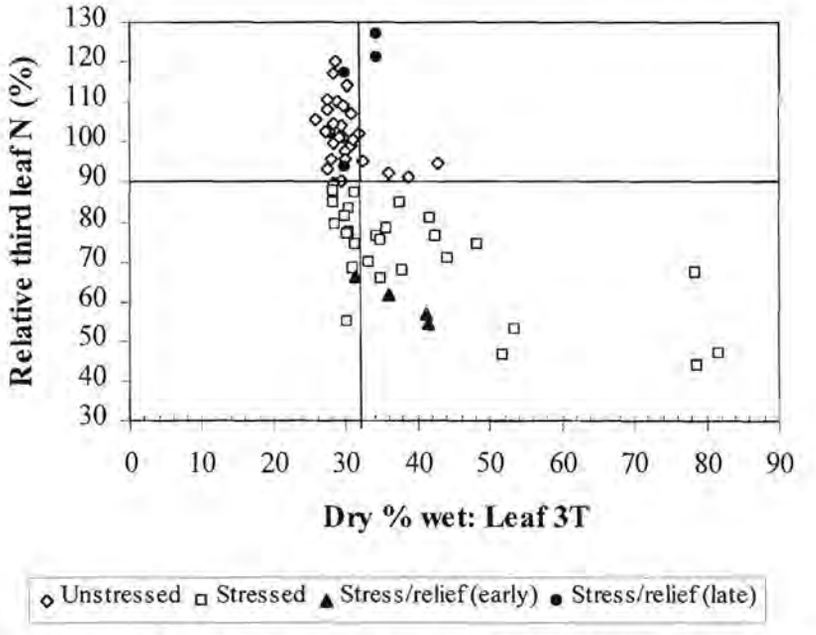

Figure 7.2. Relative third leaf N (%) plotted against D%W (L3T). D%W value of below 32% would indicate unstressed conditions in the third leaf.

The differential increases in D%W values of the spindles and third leaves associated with the relief of moisture stress indicated that various plant parts take varying times to recover from stress after re-watering. The relatively rapid recovery in moisture content of the spindles was not reflected in an increase in third leaf N. Hence the use of D%W (spindle) by itself would not be considered a suitable moisture stress index for use with leaf analysis. On the other hand, the decline in D%W values observed in the top section of the third leaf (but at a slower rate than that of the spindle) appeared to allow recovery in the third leaf N after stress relief. In order to ensure that third leaf N samples were unaffected by moisture stress, both the D%W (spindle) and the D%W (L3T) values should be below their respective critical values ie. 22 and 32% respectively (Figure 7.3)

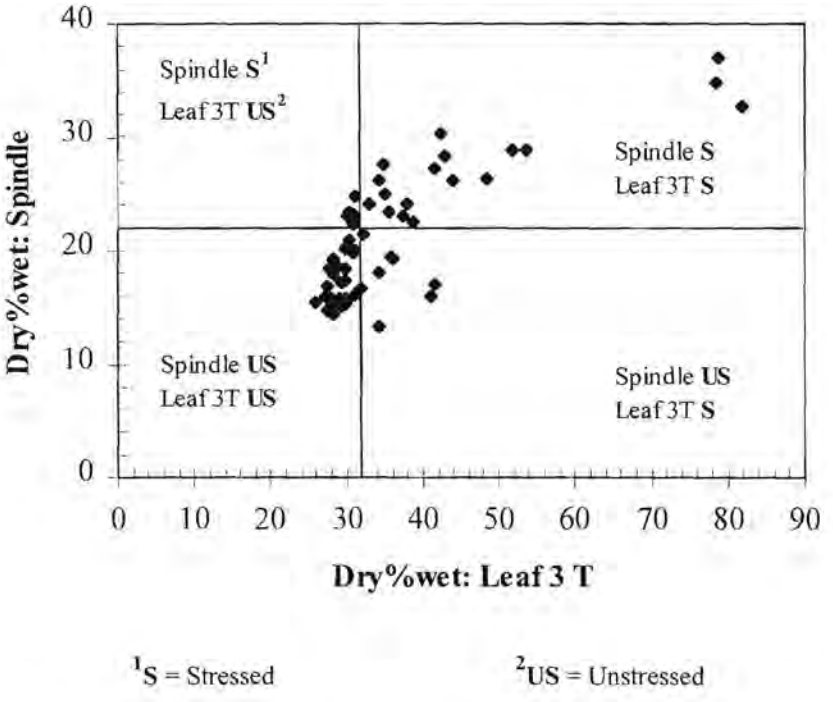

Figure 7.3. Plot of D%W (spindle) against D%W (L3T) indicating that both the D%W (spindle) and the D%W (L3T) values should be below their respective critical values ie. 22 and 32% respectively.

For validation purposes, the D%W (spindle) and D%W (L3T) associated with Trials 2 and 2(Qld) were superimposed onto the graph in Figure 7.3 (Figure 7.4). Close agreement between the data set indicated that the use of the established critical values (D%W (spindle) = 22 and D%W (L3T) = 32) were applicable to the other circumstances and varieties other than NCo376 on which they were established.

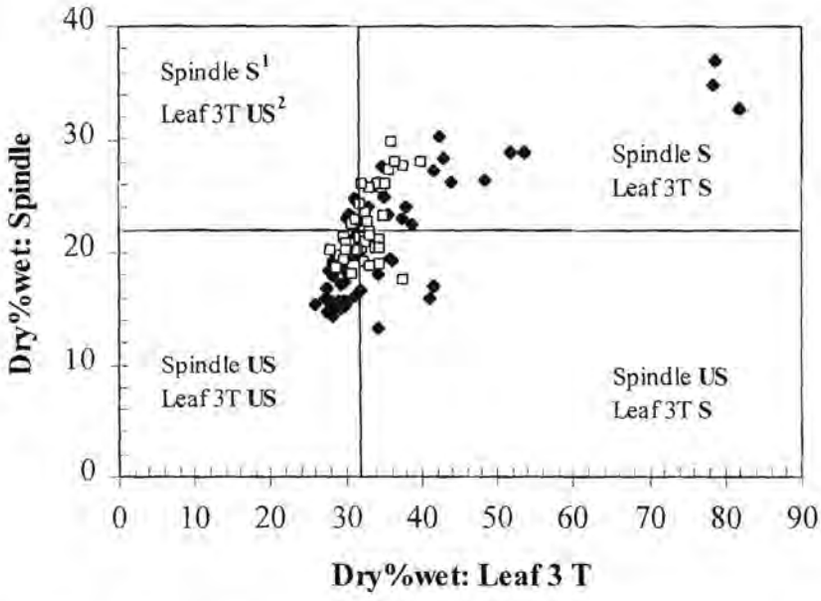

Figure 7.4. Validated plot of D%W (spindle) against D%W (L3T) indicating that both the D%W (spindle) and the D%W (L3T) of 22 and 32% respectively are suitable for general use with sugarcane.

It was found that the combination of stressed spindles with unstressed third leaves did not occur (Figures 7.3 and 7.4). This may have been expected, as stress would probably affect the moisture content of the immature parts of the plant sooner than the more stable and fully expanded third leaves.

Mean relative third leaf N (%) values (Trial 1) plotted as a function of D%W: L3T (Figure 7.5) showed that the two quantities were reasonable well correlated (r^2 = 0.656), and that the resulting regression equation (Equation 2) provided a means of determining the relative third leaf N value for a given D%W(L3T) value. In addition

the calculated relative third leaf N value would enable the estimation of an unstressed third leaf N value from a third leaf N value affected by moisture stress, as shown in the example below.

Relative third leaf N(%) = -2.16 x D%W(L3T) + 161.75 ..... (Equation 2)

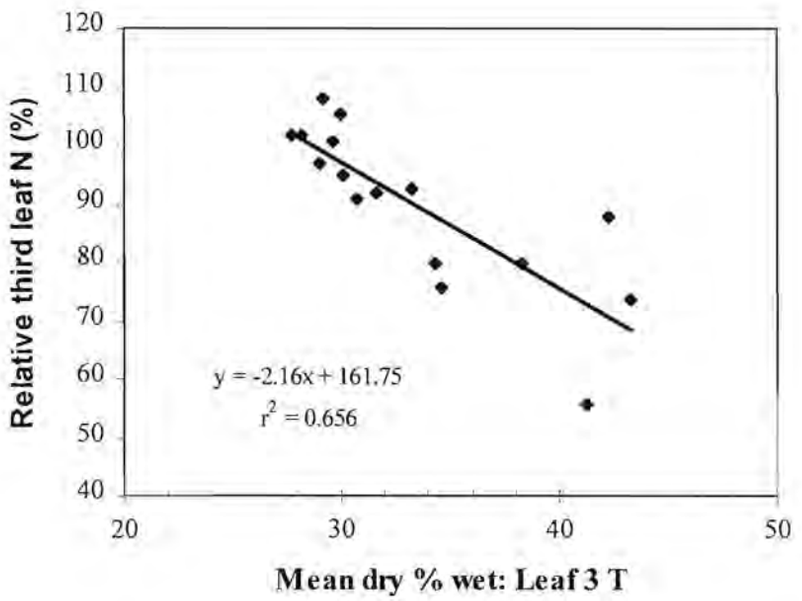

Figure 7.5. Relationship between relative third leaf N (%) and mean D%W (L3T).

Example:

Third leaf N value = 1.44%
D%W (spindle) = 25%
D%W (L3T) = 36%

- Using Figure 7.4, it is established that the sugarcane is affected by moisture stress.
- From Equation 2, it is calculated that the relative third leaf N(%) associated with a D%W (L3T) of 36% = 84%.
- The corresponding unstressed third leaf N value = 1.44 (100/84) = 1.71%

When the model (Equation 2) was tested using data from Trials 2 and 2(Qld), the resulting correlation coefficient (r^2) of predicted unstressed third leaf N values versus actual unstressed third leaf N values was in excess of 0.67 (Figure 7.6).

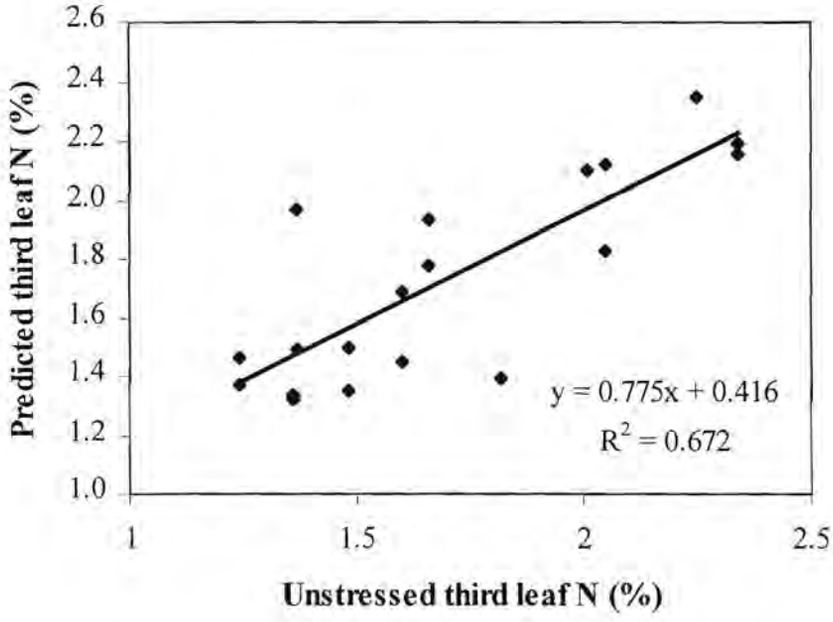

Figure 7.6. Predicted unstressed third leaf N (%) values plotted against actual unstressed third leaf values from Trials 2 and 2 (Qld).

In a similar way, it was found that D%W (L3T) could be used to establish relative third leaf P values of sugarcane affected by moisture stress, and hence estimates of third leaf P values (if stress had not been present). However, as plant P has been found to be less sensitive than N to moisture stress (Chapter 5), the relationship (Equation 3) based on Trial 1 data, was found to be relatively weak (Figure 7.7) with an r^2 value of 0.317.

Relative third leaf P(%) = -1.356 x D%W(L3T) + 132.67 ..... (Equation 3)

However, a comparison of actual and predicted 'unstressed' third leaf P values gave a correlation coefficient (r^2) of 0.506 (Figure 7.8).

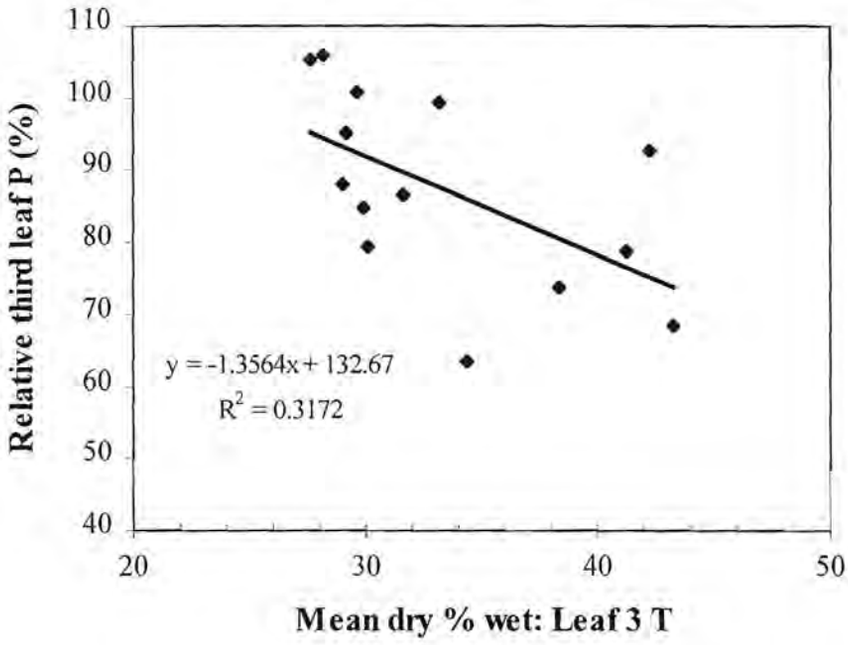

Figure 7.7. Relationship between relative third leaf P (%) and mean D%W (L3T).

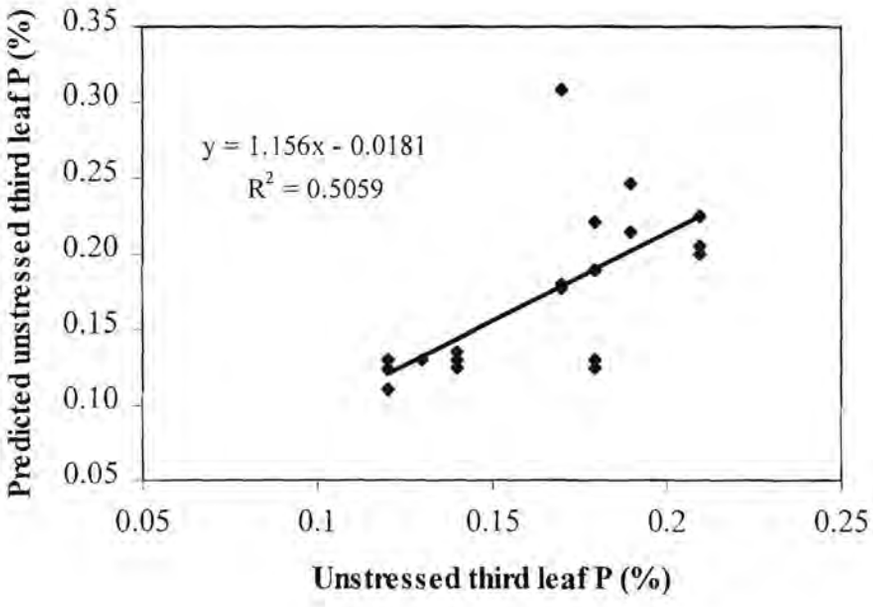

Figure 7.8. Predicted unstressed third leaf P (%) values plotted against actual unstressed third leaf P values from Trials 2 and 2(Qld).

7.4. Conclusions

The following conclusions were drawn:

- D%W (L3T) used in combination with D%W (spindle) was found to be a suitable method for determining whether sugarcane was affected by moisture stress at the time of leaf sampling.
- The established critical values of 32% and 22% for D%W(L3T) and D%W (spindle) respectively appeared to be both robust and generally applicable irrespective of variety, cane age at sampling or sampling date.
- The use of D%W (L3T) and D%W (spindle) enabled easy detection of moisture stress, and allowed assessment of whether any previous moisture stress effects had dissipated, particularly in relation to the effect on third leaf nutrient values.
- For sugarcane to be deemed unstressed (in terms of the effect on third leaf nutrient values) both D%W (L3t) and D%W (spindle) need to be below the critical values.
- This method can be easily incorporated into routine leaf sampling procedures (Appendix B), as growers will easily be able to place the previously discarded top sections of the third leaf samples (undried) into a plastic bag for submission to the laboratory for moisture determination. Likewise undried spindle samples will need to be collected at the same time as the third leaf samples.
- Apart from the identification of moisture stress effects, this assessment has also resulted in a method for estimating unstressed third leaf N values from third leaf N values affected by moisture stress. Although the regression equation for third leaf N values may be used with more confidence than that of the third leaf P values, both relationships offer a practical tool for allowing interpretation of nutrient values affected by moisture stress. This would be done by comparing the estimated unstressed values with the established third leaf critical values (Table 1.1).

General discussion and conclusions

The fact that leaf analysis continues to be a widely used for diagnostic and advisory purposes is evidence that it is considered a very useful tool in determining and monitoring the nutrient levels in sugarcane production. Despite ongoing development and re-assessment of nutrient critical values to ensure that varietal, climatic and other factors are taken account of, there has been continued concern about the interpretation of leaf analysis data that may possibly have been affected by moisture stress.

The evidence presented in this dissertation highlighted the fact that misinterpretation of leaf analysis data associated with moisture stressed sugarcane is indeed possible. As moisture stress has been shown to affect both total and third leaf N and P values, induced nutrient 'deficiencies' due to drought effects could easily be mistaken for nutrient deficiencies *per se*. Although the avoidance technique of precluding the collection of leaf samples during periods when cane is affected by moisture stress has in the past served to ensure that interpretation of leaf analysis data is as meaningful as possible, it has not been totally satisfactory. Not only do sampling restrictions hinder on-farm activities, but latent moisture stress effects may result in misdiagnosis of nutrient problems and/or erroneous fertiliser recommendations.

This investigation, conducted under semi-controlled conditions, was aimed at quantifying the response of young sugarcane to moisture stress and stress/relief and then relating this to third leaf nutrient values in particular. In this way, it was shown that the original hypothesis (derived from anecdotal evidence) of declining third leaf N values associated with moisture stress, followed by substantial improvement once the stress was relieved, was indeed valid. However, the delay in recovery of the third leaf values once stress was relieved suggested that N (absorbed into the stalk under stressed conditions) was redistributed to the younger actively growing spindle and young leaves rather than to existing fully expanded leaves. As such it would be necessary to ensure that the existing spindle (at the time of stress relief) developed to the third leaf stage before leaf sampling was conducted. Although the arbitrary chosen four week waiting period, advocated in the past in this regard, was a reasonable

estimate to allow for moisture effects to dissipate, a newly developed moisture stress indicator would need to be less subjective. The proposed use of D%W(Sp) and D%W (L3T) provides such an opportunity and has the added value in allowing the estimation of 'unstressed' third leaf N (and P) values corresponding to third leaf values affected by moisture stress.

This investigation has also shown that total and third leaf N, P and K values are differentially affected by moisture stress and stress/relief. While P is less sensitive than N to changes in plant moisture status, plant K was generally found to be insensitive to moisture stress. These findings, together with the fact that no evidence was found to support the hypothesis that moisture stressed cane would preferentially absorb K, have important implications for interpreting leave analysis data associated with moisture stressed cane. The use of nutrient ratios would not appear to be useful under such conditions.

Although a conscious effort was made to select varieties that apparently differed in their tolerance to moisture stress, there was no indication that such differences existed in terms of their nutrient status under moisture stress or stress/relief. As such, it should be assumed, until shown to the contrary, that the trends in nutrient status and the use of D%W (L3T) and D%W (Sp) for determining the extent of moisture stress in cane at the time of leaf sampling are applicable for use across varieties.

Whether, leaf analysis is simply used for diagnostic purposes or for more advanced techniques in determining nutrient requirements and/or trends, it is considered extremely important that any moisture stress effects are recognised. As such, the work reported here has provided one more step in the overall progression towards a more versatile and robust use of leaf analysis in sugarcane production. The better understanding of the effect of moisture stress and stress/relief on plant nutrient status that has resulted from these investigations will allow more confidence in the interpretation of leaf analysis data irrespective of moisture stress effects. In the event of acceptance of the proposed use of D%W in routine leaf sampling, the constraints in sampling periods will be markedly eased. It is hoped that not only will these advances encourage ongoing leaf sampling in the South African sugar industry, even in seasons when drought effects would normally have curtailed such operations, but will also

stimulate renewed interest in leaf sampling for better nutrient management in countries such as Australia. Any advances that widen the scope of a particular nutritional tool can only be of benefit to the grower community and world sugar industries as a whole

List of colour photographic plates

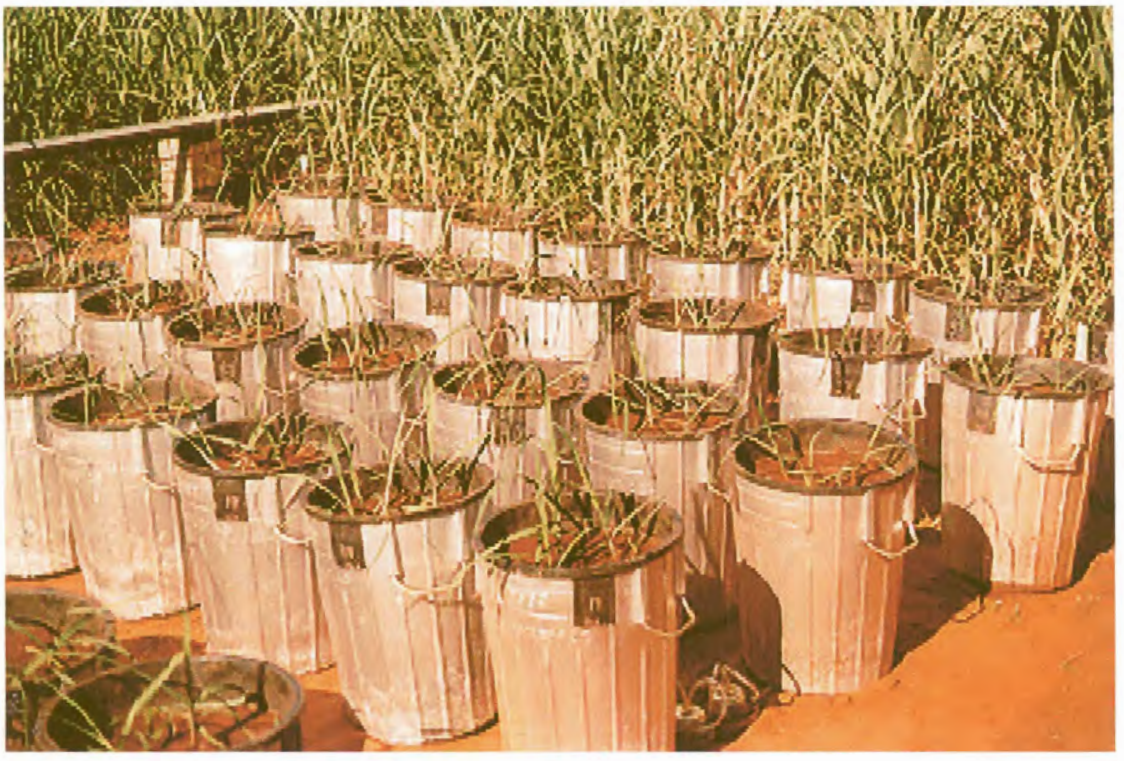

Plate 1. Sugarcane was grown in 80 litre containers.

Plate 2. Rain-shelter at the SASEX Central Field Station near Umhlanga Rocks.

Plate 3. Each pot was independently linked to a vacuum trap.

Plate 4. Growth transducers were used to measure plant extension rate.

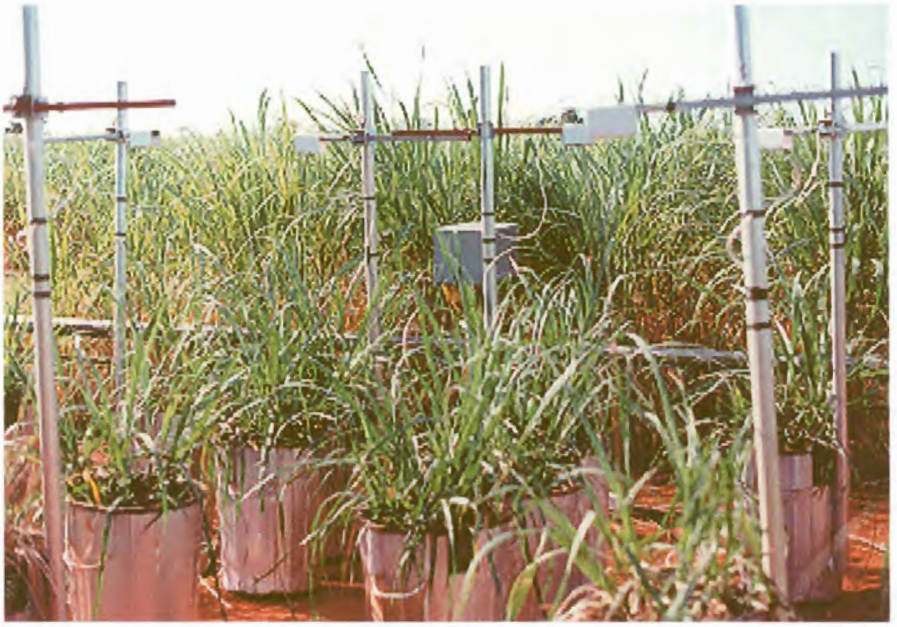

Plate 5. Plant extension rate, as well as LAI and dry matter production were determined for unstressed plants.

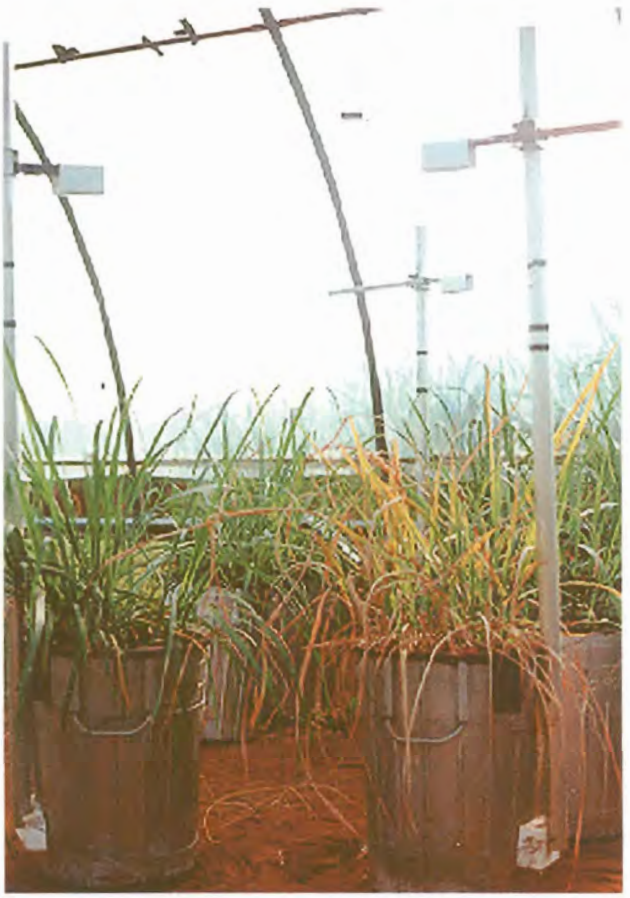

Plate 6. Plant extension rate, as well as LAI and dry matter production were determined for plants affected by moisture stress.

Plate 7. Plants were partitioned into different plant parts (spindle, leaves, sheathes, trash and stalk (if present).

Plate 8. Three different sugarcane varieties (NCo310, Q141 and Q136) were included in the glasshouse trial at Indooroopilly.

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List of Appendices

Appendix A.

Standard methods for chemical analysis of sugarcane leaves

Sample preparation:
Leaf/plant samples are dried in a forced-draught oven at 70°C for 48 hours. The dried material is finely ground using a stainless-steel hammer mill, passed through a 0.5mm perforated screen and stored in sealed containers until they are analysed. If delays occur, the samples are redried in the oven.

Analysis (SASEX):
A single wet digestion with selenised sulphuric acid is used for the determination of N, P and K.
Method of digestion:
0.25g of milled plant material is weighed on a square of tissue paper, which is then folded and transferred to a 100ml Kjeldahl flask. 2.0ml of selenised sulphuric acid and a few glass beads are added. The flasks are heated in a block digester and boiled for a minimum of 1.25 hours or until the digestion is complete. The contents of each flask is allowed to cool and is then quantitatively transferred into 25ml volumetric flasks, and made up to volume with deionised water.

Nitrogen in the digest is determined colorimetrically, using a nitroprusside catalysed indophenol reaction and reading absorbance at 665nm (Burrows, 1977).
Phosphorus in the digest is determined colorimetrically, using a phosphomolybdate complex reduced with stannous chloride and reading absorbance at 660 nm (Burrows and Meyer, 1976).
Potassium in the digest is determined by atomic absorption spectroscopy after the addition of lanthanum oxide.

Analysis (BSES):
Two separate digestions are used - one for N and a separate digestion for P and K.
Nitrogen is determined using a semi-micro Kjeldahl digestion in concentrated sulphuric acid (in the presence of selenium) similar to that described above, followed by auto-colorimetry using the indophenol method (Warner and Jones, 1970; Chapman and Haysom, 1984).

P and K are determined by ICP following digestion using a mixture of nitric and perchloric acids.
Method of digestion (Chapman and Haysom, 1984): 1.0g of milled plant material is weighed into a 125ml erlenmeyer flask. A few glass beads and 10ml of nitric/perchloric acid mixture is added. After the initial reaction has subsided, digested continues at 250°C. Once the reaction is complete, the contents of the flask is quantitatively transferred to a 50ml volumetric flask and made up to volume with deionised water.

Phosphorus is determined in the digest using ICP (214.914nm emission line).
Potassium is determined in the digest using ICP (766.491nm emission line)
(Z.A. Ostatek-Boczynski - pers. comm.)

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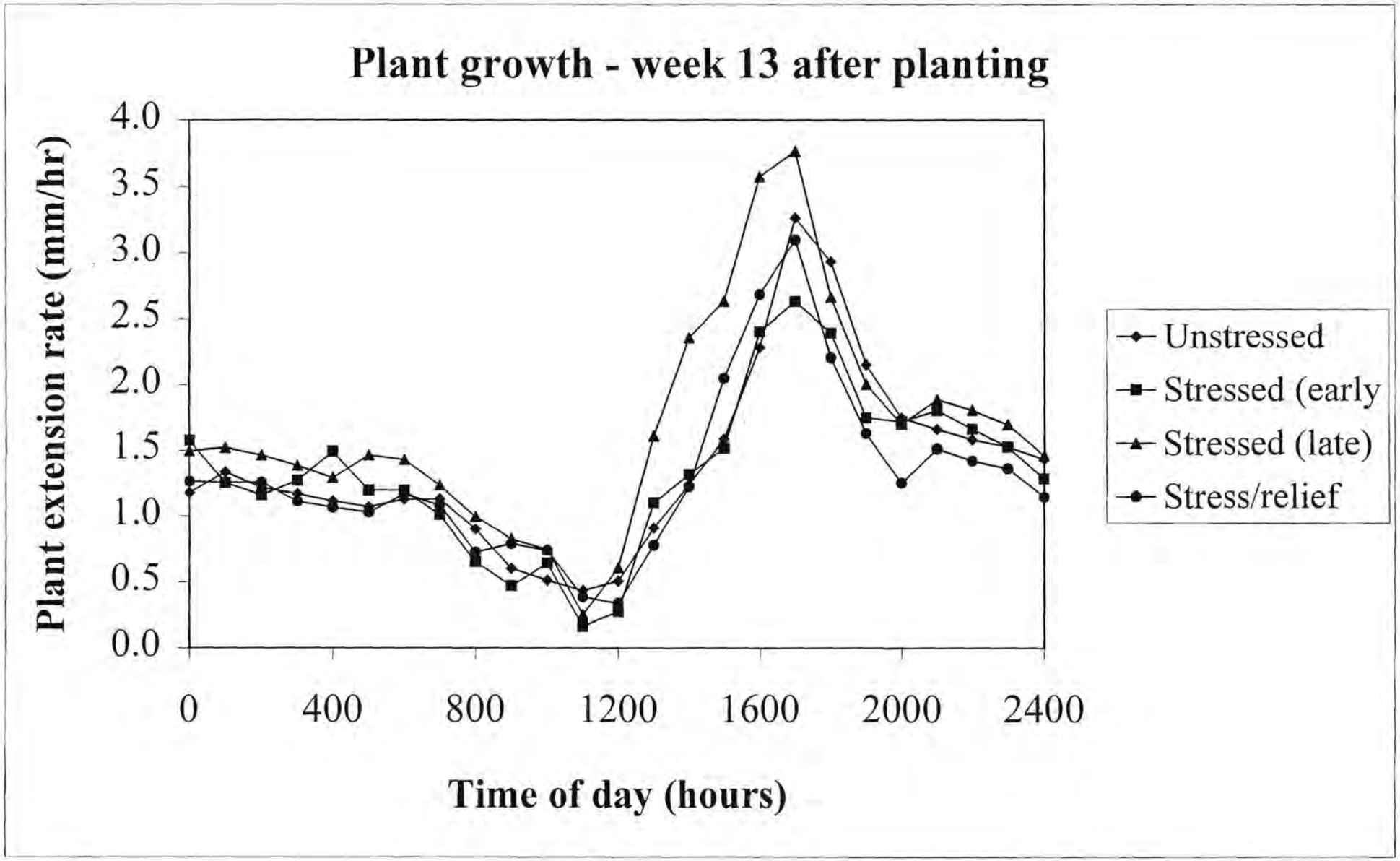
Appendix B.

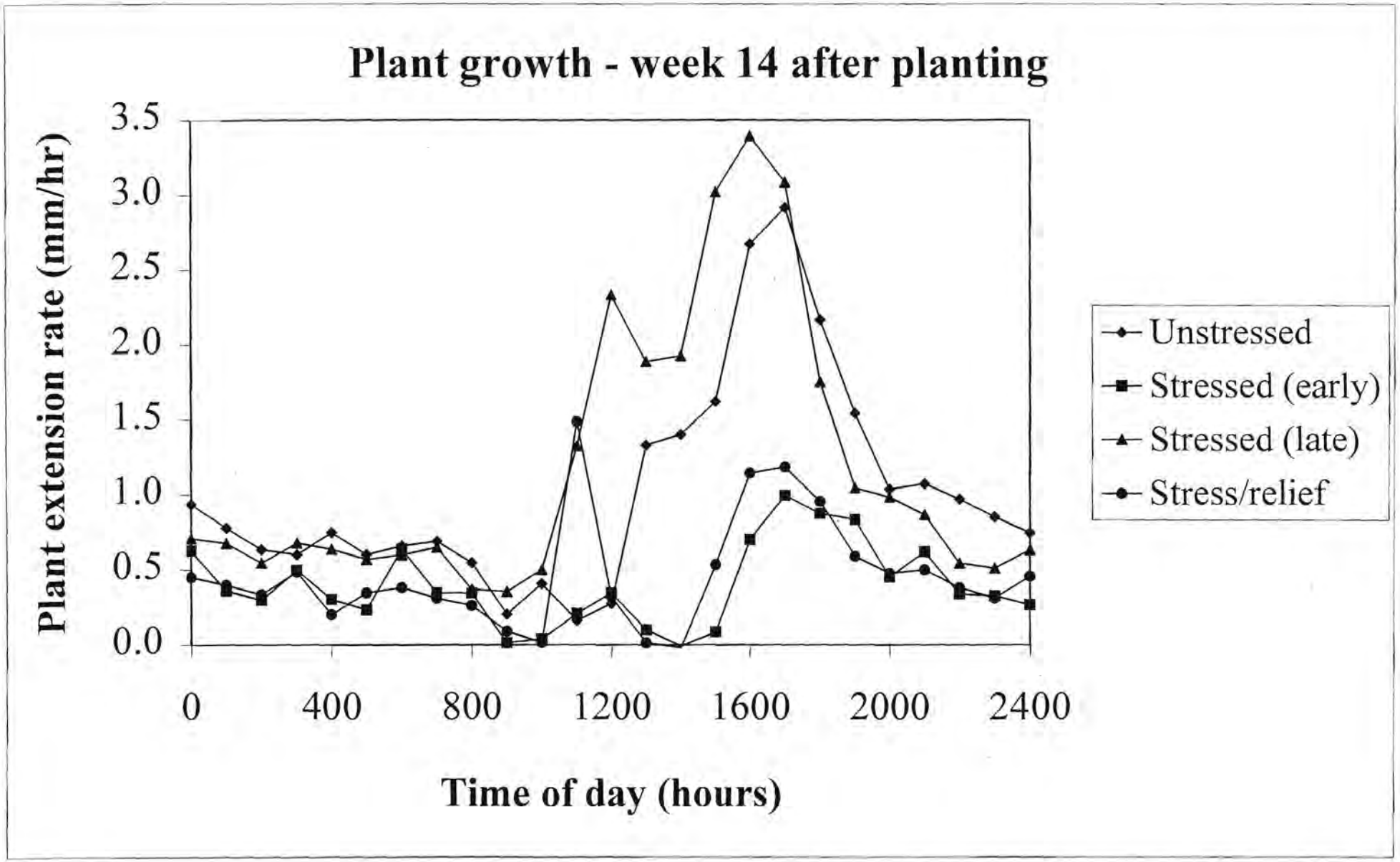
Hourly plant growth patterns associated with the different moisture treatments during a 24-hour period prior to each sampling (harvest) in Trial 1.

Appendix B(i)	Period 1: prior to imposition of the stress treatments (week 13 after planting).
Appendix B(ii)	Period 2: prior to first sampling (week 14 after planting).
Appendix B(iii)	Period 3: prior to second sampling (week 15 after planting).
Appendix B(iv)	Period 4: prior to third sampling (week 17 after planting).
Appendix B(v)	Period 5: prior to fourth sampling (week 18 after planting).

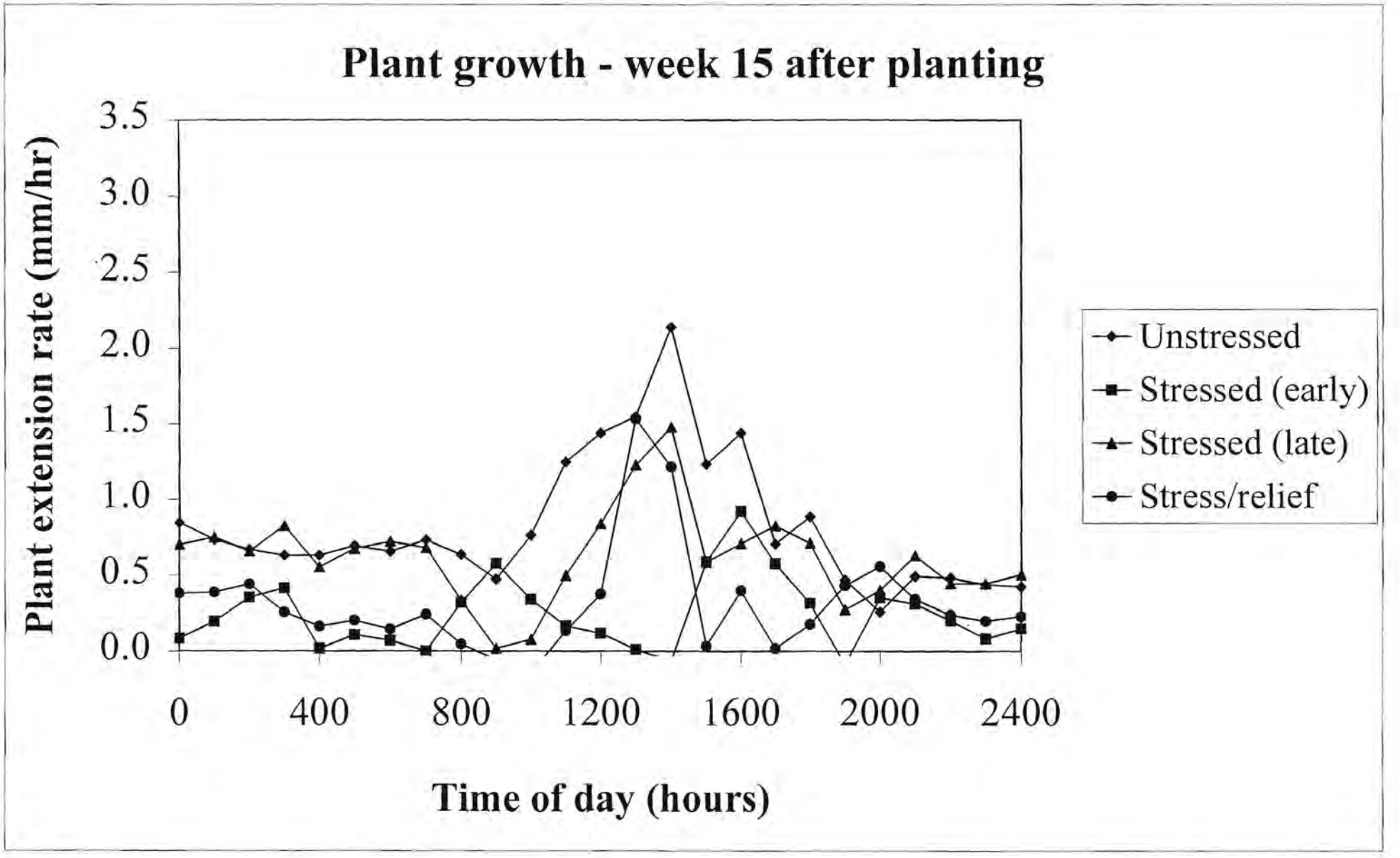
Appendix B(i)


Plant growth - week 13 after planting
Plant extension rate (mm/hr)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
400
800
1200
1600
2000
2400
Time of day (hours)
Unstressed
Stressed (early
Stressed (late)
Stress/relief

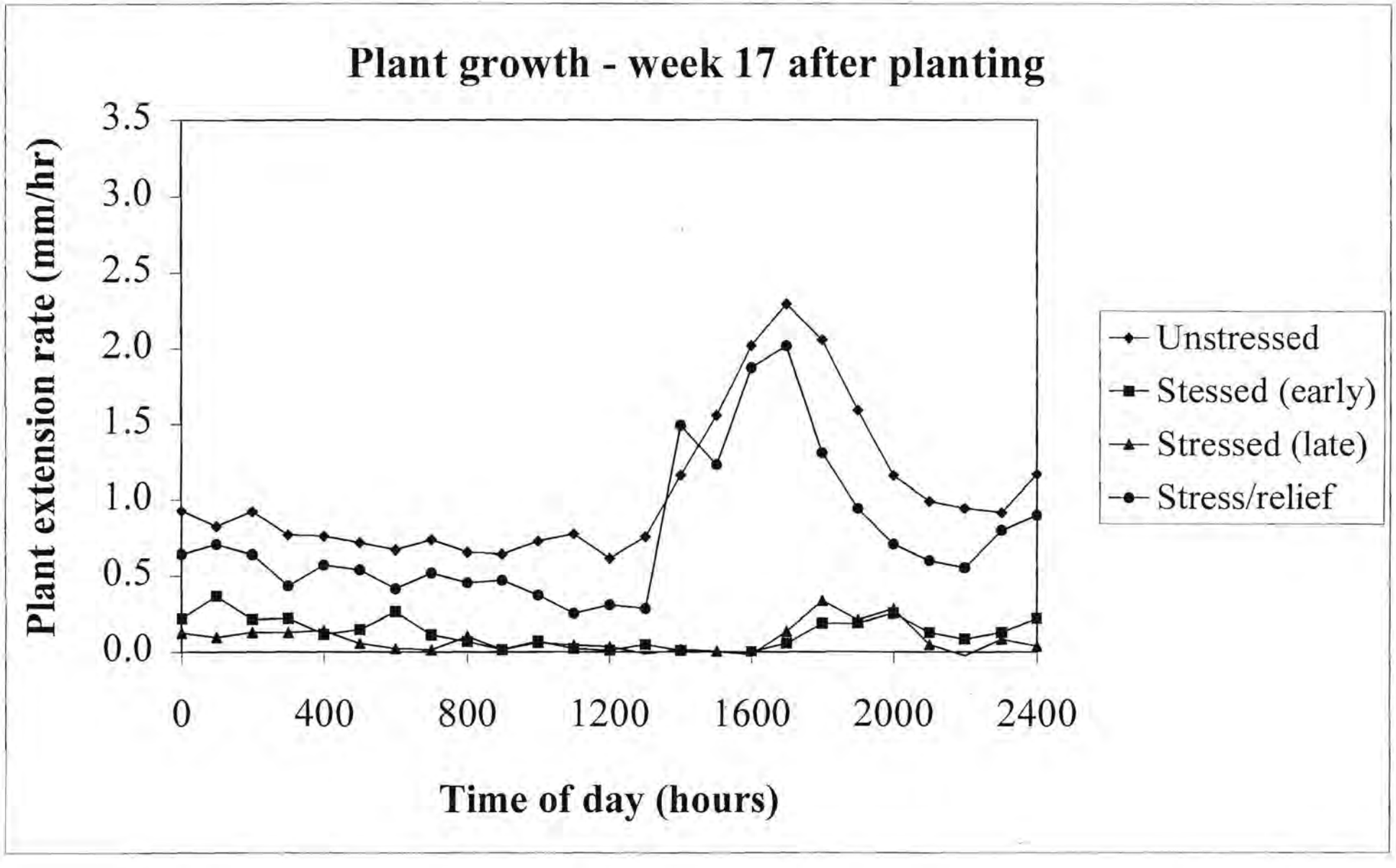
Appendix B (ii)

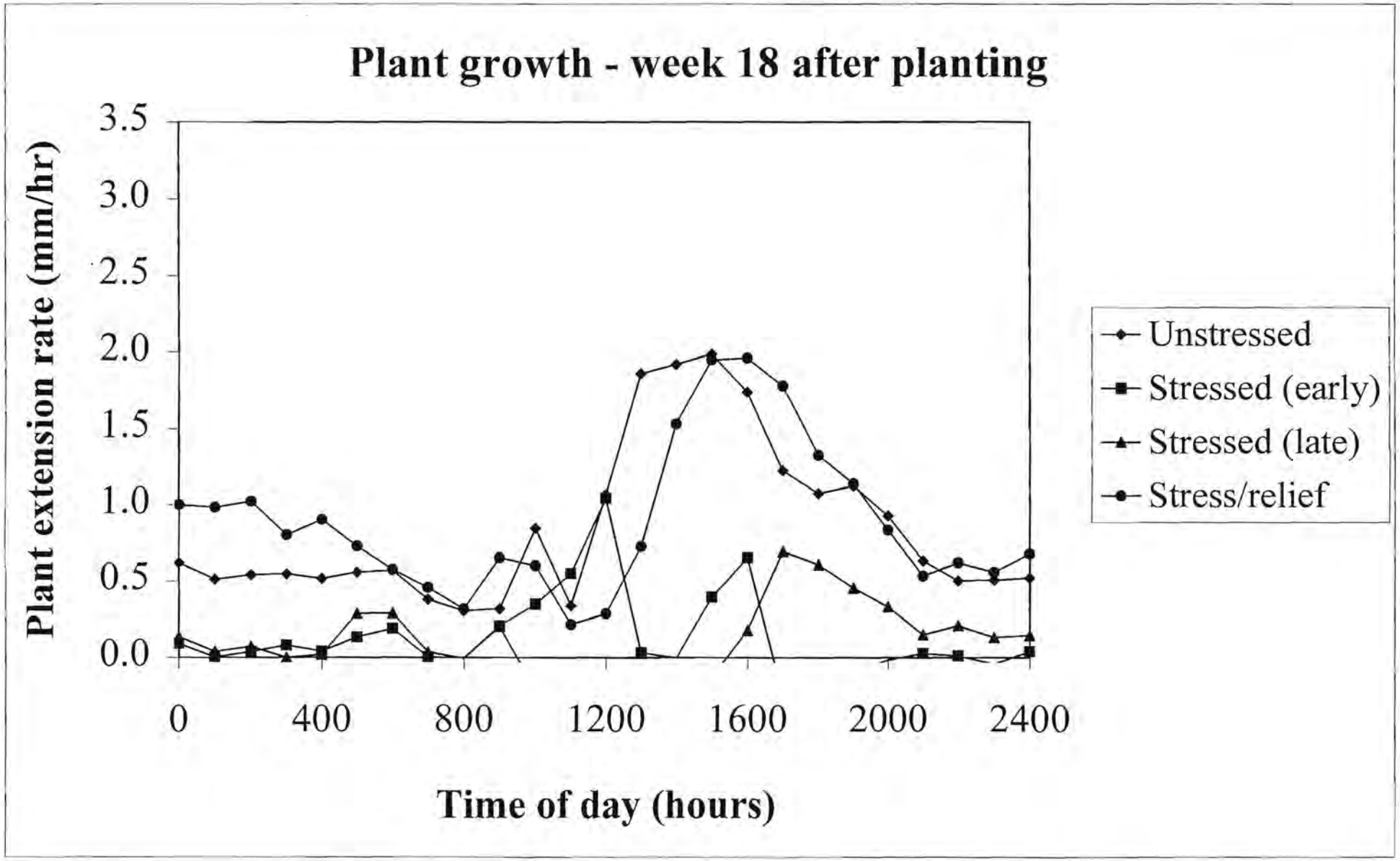


Appendix B (iii)



Appendix B (iv)





Appendix C

Recommended leaf sampling procedure

- Select leaves from stalks of average height.
- Sample the third leaf from the top of the stalk. The first leaf is the one that is more than half unrolled.
- Collect 40 such leaves from the block / field of sugarcane, preferably using a diagonal sampling pattern.
- **If it is suspected that the sugarcane may be affected by moisture stress**, collect about 20 spindle leaves from the same stalks and place directly into a plastic bag and seal it as soon as possible once the sampling is complete.
- Fold the third leaves in half (tip to base) and cut a 100mm length from the folded leaf (giving a total of 200mm).
- Strip out and discard the midrib from the 200 mm section.
- Place the sample in a clean paper bag.
- **If it is suspected that the sugarcane may be affected by moisture stress**, collect the top-sections of the leaves (above the removed 200mm section) and place in a clean plastic bag (preferably with a zip top). Seal this bag a soon as possible.
- Keep all the samples in a cool environment (polystyrene cooler) until the 200mm sections of the third leaves can be dried in an oven, or in a well-ventilated area.
- Do NOT dry the spindle or tops of the third leaves (keep them sealed in their respective plastic bags).
- Send the samples to the laboratory for analysis as soon as possible. The moist samples (spindle and third leaf tops) should be dispatched within 24 hours samples (preferably by courier or post) to prevent the growth of mould in the plastic bags.
- Supply the following information:
  - Name, address and mill area.
  - Block/field number
  - Variety
  - Crop (plant or ratoon number)
  - Sampling date and age of cane at sampling
  - Details of fertiliser applied (type and rate)
- Always ensure
  - Requirements for sampling in terms of season, age, time lapse from fertiliser applications are met
  - Hands are clean when sampling
  - Cane is not affected by some other factor such as disease, insect damage or abnormal climatic factors. Interpretation of third leaf data samples from sugarcane slightly affected by moisture stress is possible as long as the spindle and third leaf tops are sampled as well.
  - Samples are not contaminated by fertilisers and/or other chemicals

Supplement to the thesis

Published papers associated with the thesis "Assessment of leaf analysis and the major nutrient content of sugarcane under moisture stress conditions"

Contents
(in chronological order)

Schroeder, B.L., Wood, R.A. and Meyer, J.H. (1992). Advances in leaf analysis techniques and interpretation in the South African sugar industry. Proc. Int. Soc. Sugar Cane Technol. 21: 123-135.

Schroeder, B.L., Wood, R.A. and Meyer, J.H. (1993). Foliar analysis in the South African sugar industry for diagnostic and nutrient trend purposes. In N.J. Barrow (ed.). Plant nutrition – from genetic engineering to field practice: 299-302. Kluwer Acedemic Publishers, Dordrecht, The Netherlands.

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ADVANCES IN LEAF ANALYSIS TECHNIQUES AND INTERPRETATION IN THE SOUTH AFRICAN SUGAR INDUSTRY

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ABSTRACT

For many years leaf analysis has served as an important diagnostic tool in the sugar industry in South Africa. The use of X-ray fluorescence spectroscopy and near infra-red reflectance have largely eliminated the need for routine wet chemical procedures for foliar analysis. Several useful applications have been developed based on these techniques, including the establishment of a data base for trend analysis within the industry. More meaningful interpretation of results in now possible due to the recognition of the effects of varietal differences, climatic conditions and soil properties on leaf nutrient values. In particular, the leaf threshold values for P and K in terms of varieties N12 and N14 respectively have been modified and a correction factor for the leaf K threshold value for winther cut cane has been introduced. Possible alternatives for countering the effects of moisture stress on leaf N and P values are suggested.

Key words: Leaf analysis, X-ray fluorescence spectroscopy, near infra-red reflectance, threshold values.

INTRODUCTION

The primary objective of the Fertilizer Advisory Service (FAS) which was established by the South African Sugar Association Experiment Station in 1954, is to provide growers with whole cycle fertilizer advice based on the analysis of soil samples taken prior to planting. Recommendations are made for the plant and four successive ratoon crops. Leaf analysis of ratoon cane is subsequently used for evaluating the adequacy of the original advice based on soil analysis. The use of foliar analysis as a diagnostic tool in the South African sugar industry was originally evaluated in the fifties (Du Toit[7]). The methods of analysis which were based on the chemical digestion of leaf tissue were further developed and improved (Bishop[1], Long[11], Burrows and Meyer[4], Burrows[3]) until the introduction in 1984 of X-ray fluorescence spectroscopy (Wood *et al*[24].) and near infrared reflectance (NIR) (Meyer[14]) as the routine methods of foliar analysis. X-ray fluorescence spectroscopy (XRF), although widely used in the geological and metallurgical fields for the

determination of a range of elements in rock and ore samples (Jenkins and de Vries[9], Willis[22]) and also reported as a technique for foliar analysis overseas (Jenkins *et al*[10], Norrish and Hutton[18]) is not commonly used in agricultural laboratories in South Africa.

A brief resumé of the development of the leaf analysis techniques used by FAS is given together with some of the advantages of the non-destructive procedures over the conventional chemical methods. Some useful applications of foliar analysis are discussed and improvements in the understanding and interpretation of results are highlighted.

METHODS

Chemical methods

Prior to the purchase of the X-ray spectrometer all leaf samples were analysed after chemical digestion of leaf tissue. To facilitate the determination of both macro- and micro- elements, two separate digestions were necessary. One using selenized sulphuric acid for N, P, K, Ca and Mg, and the other, using a mixture of concentrated nitric and perchloric acids for Zn, S, Mn, Cu and Fe. The analytical procedures originally included techniques such as flame photometry for K, Ca and Na, colorimetric determinations for P, Mg, Zn, Cu and Mn and a distillation/titration procedure for N (Bishop[1]). The semi-automated analytical equipment which was introduced into the laboratory in 1975 eliminated the manual recording of reading from hand operated instruments with no loss in accuracy or precision (Burrows and Meyer[4]). In addition it was found that this instrumentation provided the opportunity of replacing the extremely time consuming N determination with a colorimetric procedure (Burrows[3]). These advances in methodology although contributing to a more efficient system, still relied on the digestion of samples which is time consuming and hazardous.

Non-destructive techniques

The introduction of XRF spectroscopy as a technique for analyzing leaf samples was made possible once it had been established that NIR could be used effectively for the determination of leaf N values, as nitrogen is too low on the periodic table for analysis by XRF. An integrated approach based on these two techniques was first described for sugarcane by Wood *et al*[24]. X-ray fluorescence spectroscopy is based on the principle that sample matter irradiated with X-rays will emit secondary or fluorescent X-ray wavelengths of which are characteristic of the elements within the sample. The intensity of these secondary X-rays, once dispersed into individual wavelengths, will indicate the concentration of the constituent elements present. The instrumentation currently used in the laboratory consists of a Philips sequential

semi-automatic X-ray spectrometer and a Hewlett Packard computer for calculating and printing results (Schroeder *et al*[10]). Near infrared spectroscopy, on the other hand, uses the intensity of reflected radiation as a measure of the constituent protein (or N) in a sample. The reflected light is inversely proportional to the energy absorbed at a particular wavelength. This absorbed energy coincides with the vibrational energy of the molecules composing the sample (Meyer[14]). Two Technicon NIR spectrometers are currently used for this purpose. The XRF and NIR techniques, apart from being non-destructive, are both rapid and compatible with future laboratory automation. In an initial assessment, it was found that these methods of analysis produced results which were comparable with those obtained by the colorimetric and atomic absorption procedures with little loss in accuracy or precision (Wood *et al*[24]). Reproducibility of the methods was found to be extremely precise for K and Ca (CV less than 5%) and while less precise for P, Mg, S and Zn (CV of 7 – 9%), nonetheless acceptable.

PROCEDURE

Sample preparation

Third leaf samples randomly collected from a field or plot are stripped of their midribs. The middle 300 mm of the laminae are dried in a forced draught oven at 70°C for 48 hr. The dried material is finely ground and passed through a 0.5 g mm perforated screen. Five grams of the sample are thoroughly mixed with 3 g of binder and compacted by means of a hydraulic press into a disc at 153 megapascals pressure for 20 sec (van Zyl[25]). The discs are then stored in a desiccator prior to analysis.

Calibration and analysis

Once the instruments have been calibrated for each element over the usual operating range, recalibration is not often required. Calibrations are however checked daily using reference material before analyzing the unknown samples. The sample discs are compatible with both instruments. The N content is however determined prior to insertion into the X-ray spectrometer as the high intensity radiation appears to affect the NIR readings. In addition NIR is particularly sensitive to moisture, necessitating adequate drying before sample analysis. Whilst sample discs are individually inserted by hand into the NIR spectrometer and results printed within 15 sec, the X-ray spectrometer allows four prepared samples to be placed in the sample chamber. Each sample sequentially moves into the analyzing position and is automatically analyzed for P, K, Ca, Mg, S and Zn. This procedure takes about six min for the four samples.

APPLICATIONS

Routine leaf analysis

Between March 1990 and February 1991, over 32, 000 soil and leaf samples were received by FAS. Based on a trend analysis conducted by Burrows[3] and updated in 1990, it is estimated that this number could increase to 44, 000 per annum by the year 2000. The spread of leaf and soil samples received is generally similar each year. While the peak of the soil sampling season is reached during June, July and August, the bulk of the growers' leaves are usually received during February and March. Over 80, 000 leaf samples have been analyzed since the inception of the non-destructive techniques (Table 1). The average number of leaf samples analyzed annually over the past 7 years was 11, 450, while the average number of soil samples analyzed per year, over the same period, was 14,772. Of the total number of samples received in the 1990/1991 season, 13,451 were growers' soils and 8,091 growers' leaves. This represents a ratio of about 5:3 for the whole industry. In addition to the routine XRF and NIR analyses conducted in the laboratory, every tenth leaf sample is digested and chemically analyzed (Figure 1). Comparison of results serves as a useful quality control mechanism (Table 2).

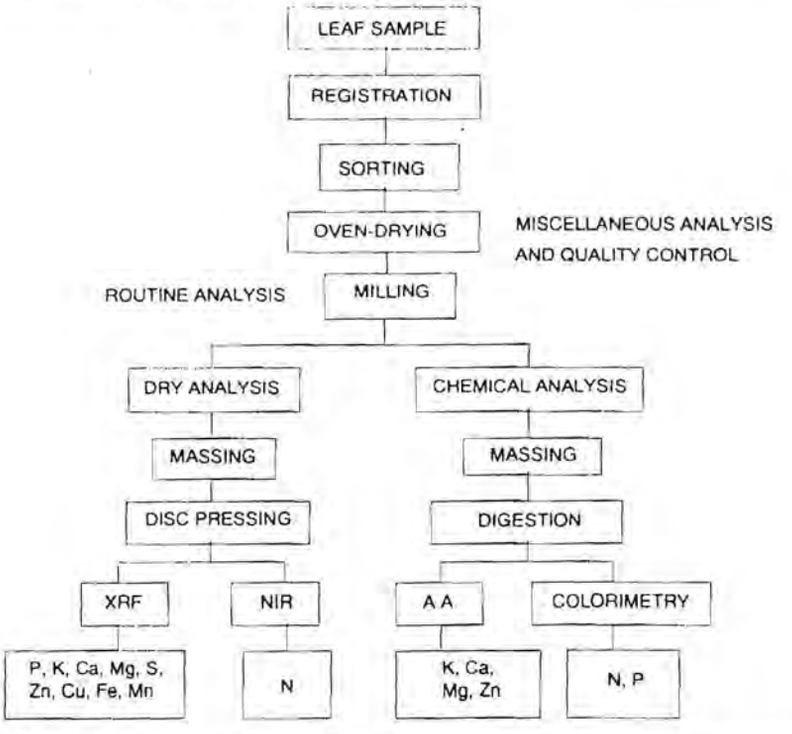

FIGURE 1. Flow diagram illustrating the current laboratory procedures for foliar analysis.

TABLE 1. Soil and leaf samples analyzed (1983 – 1991).

Season	Samples analyzed	
	Soil	Leaf
84/85	16 515	11 188
85/86	12 837	11 083
86/87	14 853	11 343
87/88	13 391	13 444
88/89	13 452	10 839
89/90	16 450	11 594
90/91	15 911	10 653
Mean	14 772	11 450
Total	103 409	80 144

TABLE 2. Comparison of XRF and NIR values with chemical digestion values of tenth sample checks.

Sample No.	Chemical digestion					NIR	Integrated system XRF			
	N %	P %	K %	Ca %	Mg %	N %	P %	K %	Ca %	Mg %
GL6840	1.72	0.20	1.34	0.17	0.13	1.68	0.21	1.37	0.20	0.15
GL6850	1.87	0.20	0.95	0.29	0.19	1.89	0.20	0.95	0.33	0.21
GL6860	1.88	0.19	1.18	0.20	0.27	1.79	0.18	1.18	0.22	0.27
GL7130	1.71	0.15	0.87	0.28	0.37	1.73	0.15	0.81	0.41	0.37
GL7140	2.24	0.21	1.61	0.28	0.16	2.12	0.20	1.55	0.29	0.18
GL7160	1.34	0.15	0.75	0.28	0.21	1.28	0.14	0.76	0.30	0.23
GL0270	1.24	0.17	1.68	0.18	0.13	1.27	0.18	1.62	0.19	0.15
GL0280	1.34	0.22	1.77	0.16	0.18	1.33	0.21	1.69	0.16	0.19
GL0290	1.05	0.17	1.52	0.15	0.12	1.15	0.18	1.54	0.14	0.14
GL0300	0.98	0.19	1.28	0.17	0.17	0.96	0.19	1.27	0.18	0.16

Non-routine leaf analysis

While all sugarcane leaf samples submitted are routinely analyzed for N, P, K, Ca, Mg, S and Zn, trace elements (Cu, Fe and Mn) are analyzed on request. The determination of all elements in the periodic table from Na to U is theoretically possible by XRF. Difficulties may however be experienced with some elements, depending on the particular X-ray tube used, in setting up instrument parameters in some instances and where concentrations are below attainable detection limits. The possible analysis of Al and Si in cane leaves was investigated in order to assist in identifying problems associated with acid soils. Du Preez[6] recognized the fact that Si plays an important role in the elimination of minor element toxicities in *Gramineae* spp. and it is thought that the use of the Mn: Si ratio may be of some value in diagnosing Mn toxicity in cane showing acid chlorosis symptoms. Further work needs to be conducted to determine whether the Al content of leaves is correlated with toxic levels of Al in acid soils.

Nutrient surveys

The computerized recommendations, first introduced by the Experiment Station in 1980, have resulted in a data bank of some 120,000 soil and more than 60,000 leaf analysis results. In a recent nutrient survey based on this information, the leaf data revealed a fairly high proportion (28%) of samples deficient in K (Meyer *et al*[17]). Approximately 13% and 12% of the samples respectively were found to be deficient in N and P, while there were relatively few deficiencies of Ca, Mg and Zn (Table 3). It was concluded that despite a 12% reduction in fertilizer usage within the industry since 1984 (Ranwell[19]), no large scale deficiencies, apart from potassium, had occurred. A micro-nutrient survey conducted throughout the industry in 1970 (Meyer, Wood and du Preez[16]) indicated that, apart from zinc no widespread trace element deficiencies were evident (Table 4). Despite the fact that this survey was based on chemical digestion procedures, the current XRF calibrations allow for similar surveys in the future.

Unfortunately XRF cannot be used for B analysis, but NIR is however showing some promise in this respect.

INTERPRETATION

Threshold values

Threshold or critical values (Table 5) have been established (Wood[23]) to assess the nutrient status of leaf samples, and include recognition of decreasing leaf N values with age (Meyer[13]). As leaf nutrient values are highly dependent on a number of factors (Bishop[1], Gosnell and Long[8]), certain conditions are specified to allow

TABLE 3. Average nutrient content of leaf samples for the various bioclimatic regions (after Meyer *et al*[17]).

Natural region	Soil system	N %	P %	K %	Ca %	Mg %	Zn ppm
Coastal lowland	Berea	1.84	0.21	1.21	0.27	0.22	18
	Umzinto	1.87	0.22	1.20	0.26	0.26	18
Coastal hinterland	Umzinto midlands	1.95	0.22	1.42	0.29	0.25	17
Mistbelt	Nottingham	1.94	0.20	1.29	0.26	0.24	18
Lowveld	Komatipoort	2.03	0.25	1.32	0.34	0.27	19
Threshold values		1.60	0.17	1.05	0.15	0.10	13
% samples deficient		13	12	28	4	1	8

meaningful interpretation of analysis results. Apart from prescribed sampling periods and cane ages (Table 5), cane must also be growing vigorously, be unaffected by moisture stress at the time of sampling, and no samples should be taken until 6 weeks after fertilizer application.

Adjusted threshold values

Specific circumstances have recently been identified where certain threshold values appear to be inappropriate. Numerous leaf analyses have indicated that K uptake by winter cut cane growing on Ca and Mg saturated clays of the northern irrigated cane areas is often depressed during much of the spring/early summer growth period despite seemingly adequate amounts of available soil potassium. Following a number of trails in which it became apparent that apart from the soil effects *per se*, the pattern of leaf K uptake was also affected by season, Ca and Mg antagonism and the prevailing soil moisture and temperature conditions. A seasonal correction factor for the leaf K threshold value (Table 5) was suggested as an interim measure for cane grown on a winter cycle under these conditions (Donaldson *et al*[1]). Although it is recognized that differences exist in the critical nutrient concentrations between cultivars (or genotypes) of the same species, these, in general, have not been rigorously examined (Smith[21]). However, two sugarcane varieties, N12 and N14, appear to exhibit lower threshold values than those of most other varieties in terms of P and K respectively (Table 5).

TABLE 4. Average nutrient content of leaf samples for various natural regions (after Meyer *et al*[16]).

Natural region	No. of samples	B	Cu	Zn	Mn	Al	Fe
		ppm					
Coastal lowland	228	4.1	6.9	18.3	48	83	146
Midlands mistbelt	135	4.0	7.2	14.9	74	133	163
Sub-humid midlands	36	2.0	6.9	17.1	67	60	103
Lowveld							
– Pongola	13	2.6	7.5	15.6	42	40	91
– Swazilan	21	4.9	8.0	18.8	25	165	196
– Transvaal	39	4.4	7.6	17.4	38	112	182
– Natal	15	3.5	6.1	23.9	35	132	173
Total	487						
Range Lowest		1.6	4.2	10.0	11	21	49
Highest		10.0	12.2	55.3	270	800	915
Threshold value		1	3	14	15	–	50
No. deficient samples		nil	nil	57	11	–	1
% of total deficient		nil	nil	11.7	2.2	–	<1

Moisture stress effects

Leaf analysis data over the past two seasons have highlighted the marked effect that moisture stress can have on leaf nutrient values. The unusual rainfall pattern experienced in 1989/90, in which below average precipitation was recorded during much of the growing season, resulted in numerous low leaf N and P values. Resampling of the same fields indicated a recovery to acceptable values once the drought effects had dissipated. Despite the fact that growers are urged to sample only when conditions will exclude moisture stress, some of the larger estates find it difficult to reschedule their programs in this manner. Future work will include the use of nutrient ratios and the development of a model for interpreting analytical results of samples taken during or after stress conditions.

TABLE 5. Leaf threshold values currently used by FAS.

Nitrogen				
Area	Crop age	Month of sampling	N (%) Plant	N (%) Ratoon
Northern irrigated	3 – 5 months	Oct to Dec	1.9	1.8
		Jan to Feb	1.8	1.7
		Mar to Apr	1.7	1.6
Coast lowlands	* 4 – 7 months	Nov to Dec	1.9	1.8
		Jan to Feb	1.8	1.7
		March	1.7	1.6
Midlands	*4 – 9 months	Nov to Dec	1.9	1.8
		Jan to Feb	1.8	1.7
		March	1.7	1.6

Other nutrients			
** P : 0.19%	Ca : 0.15%	S : 0.12%	Cu : 3 ppm
K : 1.05%	Mg : 0.08%	Zn : 13 ppm	Mn : 15 ppm

K (winter cut irrigated cane)			
Age of crop (months)	Months of sampling	K (%) All SA varieties except N14	K (%) N14
3 – 5	mid Oct – Nov	0.85	0.70
	Dec – Jan	0.95	0.80
	Feb – Apr	1.05	0.90

*In the case of summer cut ratoon cane it may be possible to sample when the crop is only three months old. For plant cane, sampling will normally only be possiblewhen the crop is five to six months old.

**The P threshold value for variety N12 is 0.03 percentage units lower than that of most other varieties.

Diagnosis and Recommendation Integrated System (DRIS)

Considerable attention was paid to evaluating DRIS for use in the sugar industry in the late seventies and early eighties (Beaufils and Sumner[2], Meyer[12]). Meyer[13] showed that this system which is based on the calculation of indices derived from nutrient ratios is particularly appropriate for young cane. This is partly due to the fact that nutrient ratios appear to vary less with increasing crop age than do the conventional nutrient percentages in leaf. As most of the cane in the sugar industry is leaf sampled when 4 months of age or older (Table 5), DRIS is not widely used except in the warmer northern irrigated areas where sampling of young cane is practiced. Under these latter conditions the system can be of benefit in providing corrective fertilizer treatment to the current crop, as N imbalances may be detected sooner than would be the case with the threshold value approach (Meyer and Wood[15]).

CONCLUSIONS

During the past 35 years, significant advances have been made in foliar analysis in the South African sugar industry. These have occurred not only in the development of the techniques and procedures used, but also in improvements of data interpretation. The system of analysis using X-ray fluorescence in conjunction with near infrared reflectance spectroscopy has been most successful since it was introduced in 1984. The rapidity of the techniques together with the reliability of the instrumentation has resulted in improved throughput of samples during the leaf sampling season. The introduction of nutrient surveys was made possible by the laboratory being able to handle a relatively large number of routine and non-routine leaf analyses. The updating and refining of leaf threshold values over the years has allowed better interpretation of leaf analysis data.

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PROGRES DANS LES TECHNIQUES D'ANALYSE FOLIAIRE ET DANS L'INTERPRETATION DES RESULTATS DANS L'INDUSTRIE SUCRIERE EN AFRIIQUE DU SUD

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RESUME

L'analyse foliaire a été utilitsée pendant longtemps comme moyen de diagnostique dans l'industrie sucrière en afrique du sud. La spectroscopie à fluorescence de rayons X et la reflectance à infra-rouges proches remplacent de plus en plus les méthodes d'analyse chimique traditionnelles. Diverses applications utiles ont été faites à partir de ces techniques, dont l'étalbissement d'une base de données qui permet de suivre l'évolution nutritionnelle de la canne à sucre. Avec l'identification des effets de différences variétales, de conditions climatiques et des caractéristiques du sol sur les niveaux de nutrients dans les feuilles, il est maintenant possible d'interpréter les résultats plus correctement. Par exemple, les niveaux critiques de P et K ont été modifiés pour les variétés N12 et N14 respectivement et un facteur de correction a été apporté aux valeurs de K pour la canne récoltée en hiver. Des moyens alternatifs sont suggérés dans le but d'éliminer les effets du stress hydrique sur les teneurs en N et P dans la feuille.

Mots clefs: Analyse foliaire, spectroscopie à fluorescence de rayons X, reflectance à infra rouge proche, niveaux critiques.

N. J. Barrow (Ed.), *Plant nutrition - from genetic engineering to field practice*, 299-302

Foliar analysis in the South African Sugar Industry for diagnostic and nutrient trend purposes.

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Key words: leaf analysis, sugarcane

Abstract

In the South African sugar industry, soil and leaf analysis are used for providing fertiliser recommendations. Leaf analysis is more accessible as a diagnostic tool since the introduction of X-ray fluorescence and near infra-red reflectance spectroscopy as the methods of routine analysis.

The effects of climate and various soil properties on nutrient uptake are now recognised as important factors when interpreting leaf analysis data. The computerisation of results over the past decade has led to the effective use of a data bank of 60 000 leaf samples. Trends in nutrient status during this period have been used to evaluate the effects of fertiliser use in different regions of the industry.

Introduction

The South African Sugar Association Experiment Station has run a fertiliser advisory service (FAS) for cane growers since the early 1950's. Fertiliser advice for a cycle of plant crop and four successive ratoons is based on the analysis of soil samples collected before planting. Analysis of leaf samples taken during the ratoons is then used to check the original recommendations. Leaf analysis, as a diagnostic tool, has achieved greater impetus since 1984 when X-ray fluorescence and near infra-red reflectance spectroscopy were introduced as routine laboratory methods. In addition, trends in nutrient uptake are monitored by means of an information retrieval system based on about 60 000 leaf analysis results.

This paper describes the use of leaf analysis for diagnostic purposes, the effect of cane variety, climate and soil properties on leaf analysis data, and the use of the information retrieval system to monitor trends in the South African sugar industry.

Methods

Sugarcane leaf samples submitted to FAS are routinely analysed for N, P, K, Ca, Mg, S, Zn, Cu, Fe and Mn by an integrated, non-destructive technique involving X-ray fluorescence and near infra-red reflectance spectroscopy (Wood et al., 1985). All elements except nitrogen are determined using a Philips 1410 sequential semi-automatic X-ray spectrometer controlled by a 386XS IBM compatible personal computer (Schroeder et al., 1990). Leaf N is determined using a Technicon 300 NIR spectrometer (Meyer, 1983). Results are categorised by a computer programme into various stages of sufficiency (very low, low, moderate, high and excessive) based on threshold values for each element. All data is stored on mainframe disc files as part of the nutrient information retrieval system (NIRS). The data for each grower also includes location, month and age of sampling, and soil type. This large data bank is used to provide information on trends in leaf nutrient values for the whole industry, or for the different regions as defined

by climatic and soil systems (Macvicar, 1973), as well as for extension areas, individual farms or fields.

Results and Discussion

Samples analysed

Computers have been used by FAS since 1980 to store analysis data of more than 140 000 soil and 60 000 leaf samples. While the bulk of soil samples are received during the winter and spring, most of the leaf samples are received during the growing season (November to March) each year. An average of about 6000 growers leaf samples have been received annually since 1980.

Threshold (critical) values

Threshold values for N, P, K, Ca, Mg, S, Zn, Cu, and Mn are based on third-leaf samples (Table 1), and have been determined from a large number of fertiliser trials conducted over the past 40 years (Du Toit, 1959; Wood, 1989). Although these are generally in terms of variety NCo376, they are used for all varieties. Leaf sampling requirements are standardised in terms sampling periods and age of cane (Table 2).

Modified threshold values

A number of factors such as age, season, time of sampling and variety, affect the uptake of elements and leaf nutrient values (Gosnell and Long, 1971). Modified threshold values have been introduced as required to take these factors into account. Examples of these are given below.

As leaf N values generally decline through the season (Meyer, 1981), modified threshold values ranging from 1.9 to 1.6 %N are used depending on cane age and month of sampling (Table 2).

With the decline in use of variety NCo367, it became apparent that variety N12, now widely planted in the higher altitude areas, had a somewhat lower leaf P threshold value than most other varieties. Variety trial data showed that, on average, the leaf P value for N12 was about 85% of the value for NCo376. A leaf P threshold value which was 0.03% P lower than the standard 0.19% P value was accepted for variety N12.

Table 1. Leaf threshold values for some elements

Macro elements		Trace elements	
Nutrient	Leaf TV (%)	Nutrient	Leaf TV (ppm)
N	1.70 (Mean)	Zn	12
P	0.19	Mn	15
K	1.05	Cu	3
Ca	0.15		
Mg	0.08		
S	0.12		

Leaf threshold values for K have been modified for two reasons. In the first case, for winter-cut irrigated cane, growing on base-saturated soils, low leaf-K values have been associated with high leaf Ca and/or Mg, low soil temperatures and moist conditions in the early part of the growing season (Donaldson et al., 1990). Consequently increasing K threshold values with month of sampling have been introduced (Table 3). In the second case, the leaf-K threshold value for variety N14 has been found to be 0.15% K lower than for other varieties.

Leaf analysis and moisture stress

Sugarcane in South Africa is largely grown under rainfed conditions. Water stress affects growth, sucrose accumulation and restricts the uptake of elements. When leaf samples were collected

Table 2. Leaf N threshold values

Area	Crop age (months)	Month of sampling	N (%)	
			Plant	Ratoon
Northern irrigated	3-5	Oct-Dec	1.9	1.8
		Jan-Feb	1.8	1.7
		Mar-Apr	1.7	1.6
Coastal lowlands	4-7	Nov-Dec	1.9	1.8
		Jan-Feb	1.8	1.7
		March	1.7	1.6
Midlands	4-9	Nov-Dec	1.9	1.8
		Jan-Feb	1.8	1.7
		March	1.7	1.6

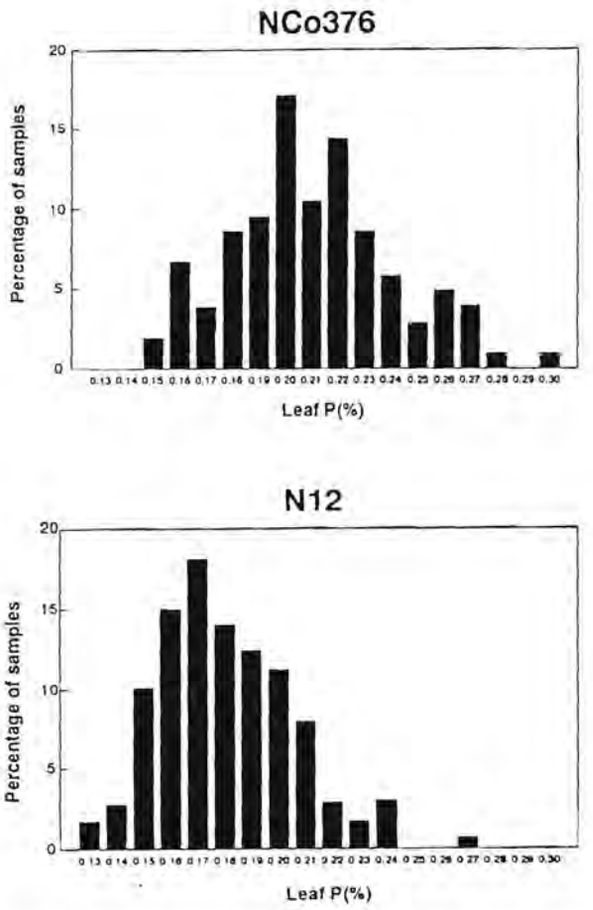

Fig. 1. Distribution of leaf P values based on samples from varieties N12 and NCo376

during moisture stress, leaf nutrient values associated with adequately-fertilised cane were often below the threshold values. An example is shown in Table 4. Rainfall for the three-month period before sampling (December 1989 to February 1990) was only 190 mm compared with the long-term average of 365 mm. Data for the February sampling showed low to very low leaf N, P and K values. By comparison, leaf samples taken in April from the same crop, after good rains (160 mm) had fallen in March 1990, indicated substantial improvement in the nutrient status despite the fact that the cane was two months older.

Nutrient trends

Apart from a micro-nutrient survey conducted in 1970 (Meyer et al., 1971), little use had been made of analytical data for examining trends in leaf data until the computerisation of FAS recommendations in 1980. The computer-based system resulted in a large data bank which is continually updated. The data (Table 5) show that there was a relatively high proportion of samples deficient in K (28%), somewhat fewer deficiencies in N (13%) and P (12%) and only infrequent deficiencies of Ca (4%), Mg (1%) and Zn (8%) for the whole industry (Meyer et al., 1989). On a regional basis, it was found that the highest incidences of N deficiency (19%) occurred in the coastal lowlands. Most P deficiencies (15%) occurred in the coastal hinterland and higher altitude areas. The majority of K deficiencies (41%) occurred in the irrigated, warm semi-arid region.

As the industry is divided up into 15 extension areas, it is useful to monitor nutrient trends in

Table 3. Leaf K threshold values for winter cut cane irrigated cane (after Donaldson et al., 1990)

Age of crop (months)	Months of sampling	Leaf K (%)	
		All except N14	N14
3-5	Oct-Nov	0.85	0.70
	Dec-Jan	0.95	0.80
	Feb-Apr	1.05	0.90

Table 4. Leaf nutrient values of samples collected from commercial cane in February and April, 1990.

Field	Variety	Ratoon	Age (mths)	N (%)	P (%)	K (%)
Sampling date: 20/2/90						
48	NCo376	5	5	**1.48	***0.12	*0.90
49	Mixed	5	5	**1.44	***0.14	**0.83
50	NCo376	7	5	***1.30	***0.12	**0.88
66	Mixed	2	4	***1.32	***0.14	1.31
Sampling date: 24/4/90						
48	NCo376	5	7	1.71	0.18	1.46
49	Mixed	5	7	1.87	0.19	1.32
50	NCo376	7	6	1.61	0.19	1.43
66	Mixed	2	6	1.76	0.20	1.52

* Marginal
** Low
*** Very low

these areas. Due to the large amounts of fertilisers applied each year, extension officers should be aware of changes in nutrient consumption and soil fertility in their areas. For instance, the most-southerly cane-growing area (Lower South Coast) in South Africa is generally confined to the coastal lowlands and coastal hinterland regions. According to the 1988 investigation, which included all leaf data from that area since 1980, about 14% of samples were deficient in N and 9% deficient in P (Table 6). These figures were close to those for the whole industry. All other nutrients appeared to be well-supplied and deficiencies were well below the industry averages. In comparison, the 1989/90 leaf data (Table 6) indicated large proportions of deficient leaf N and P values (48 and 43% respectively). These low values were probably due to moisture stress in a dry year. That the low leaf N and P values were drought-induced was subsequently supported by the 1990/91 data which indicated substantially improved values (Table 6).

Table 6. Leaf nutrient assessment - Lower South Coast extension area

Leaf nutrient	Period	Ave	Percentage of total			
			Deficient	Marginal	Adequate	High
N (%)	1980/88	1.92	14	23	60	1
	1989/90	1.69	48	23	29	0
	1990/91	1.89	15	23	62	0
P (%)	1980/88	0.22	9	28	56	4
	1989/90	0.18	43	25	32	0
	1990/91	0.19	18	35	47	0
K (%)	1980/88	1.30	3	15	59	20
	1989/90	1.18	6	25	57	12
	1990/91	1.16	3	19	76	2

Conclusions

Leaf analysis remains the most effective way of assessing the nutrient status of ratoon cane and checking on the adequacy of fertiliser recommendations. Threshold values for diagnostic purposes are continuously updated and modified as required. Although growers are urged not to leaf sample when cane is affected by moisture stress, drought is not uncommon in South Africa. Consequently there is a need to study the effects of moisture stress on leaf nutrient values. The use of nutrient ratios and/or a computer model for interpreting nutrient uptake under various moisture regimes will be considered. The development of a data bank such as the NIRS, not only serves to identify trends of diminishing fertility, but will also aid in preventing over application of fertilisers.

Table 5. Average nutrient content of leaf samples for the various natural regions (after Meyer et al., 1989)

Natural regions	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (%)
Coastal lowland	1.84	0.21	1.21	0.27	0.22	18
Coastal hinterland	1.95	0.22	1.42	0.29	0.25	17
Midlands	1.95	0.20	1.29	0.26	0.24	18
Lowveld	2.03	0.25	1.32	0.34	0.27	19
Threshold value	1.60	0.17	1.05	0.15	0.10	13
% samples deficient	13	12	28	4	1	8

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Leaf analysis—what does it offer the Australian sugar industry?
Proc. Aust. Soc. Sugar Cane Technol., 21: 122–130 **1999**

LEAF ANALYSIS—WHAT DOES IT OFFER THE AUSTRALIAN SUGAR INDUSTRY?

By

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Abstract

Although leaf analysis is routinely used in many Australian and overseas agricultural industries for nutrient diagnostic and/or advisory purposes, it is seldom used for commercial purposes within the Australian sugar industry. This paper reviews the local and international experiences relating to foliar testing in sugarcane production, and reports on investigations aimed at improving the interpretative skills associated with leaf analysis. The need for increased local usage of leaf analysis is explained, particularly in light of important potential roles within the industry. These include improved diagnostic capabilities, monitoring of nutrient trends within the industry and providing a basis for checking on the adequacy of fertiliser recommendations. Leaf sampling requirements, prerequisites and procedures are explained to ensure effective leaf analysis. It is concluded that there is a need to take account of varietal differences, age of the crop at sampling, climatic conditions and soil properties when interpreting leaf analytical data. As moisture stress can affect leaf nutrient values, it is important that strategies exist either to preclude this effect or to identify its occurrence. Renewed usage of leaf analysis within the Australian industry will not only have important implications for nutritional research and extension, but will also ensure that growers are able to make more informed decisions regarding nutrient management on the farm.

Introduction

Leaf analysis was probably first established as a useful diagnostic tool by the early chemists who recognised that relationships existed between crop yield and the nutrient content of plant ash (Hall, 1905; Liebig, 1840; Mitscherlich, 1909). Following the work of Macy (1936), which established methods for interpreting these relationships, much effort has been devoted to providing information on the nutrient status of plants with a view to managing nutrition for optimum plant production (Smith and Loneragan, 1997). This has resulted in leaf analysis being used for a number of different applications, which include:

- Diagnosis of existing problems (nutrient deficiencies, toxicities and/or nutrient imbalances);
- Prediction of nutrient problems in current (likely between sampling and harvest) or succeeding crops;
- Monitoring the crop nutrient status (effectiveness of fertiliser practice, crop removal, overall nutrient status of regions, districts, soil types, etc) (Smith, 1986; Smith and Loneragan, 1997).

Leaf analysis has, over the years, been more extensively used and/or investigated in some sugar producing countries than others. Although much effort has been directed towards establishing and/or confirming critical leaf values (Smith and Loneragan, 1997), attempts to base fertiliser recommendations directly and/or solely on leaf analysis values (Farquhar, 1965; Malavolta, 1994 citing Poidevin, 1964, and Poidevin and Robinson, 1964; Samuels, 1959) have

KEYWORDS: Leaf Analysis, Sugarcane Nutrition.

largely been unsuccessful. However, leaf analysis continues to be widely used for diagnostic purposes, as a means to check on the adequacy of fertiliser recommendations (Malavolta, 1994; Schroeder *et al.*, 1992), and to identify nutrient trends (Meyer *et al.*, 1989). Nonetheless, the current inability to interpret leaf nutrient values associated with samples collected under conditions of moisture and other stress, often results in unfair criticism of leaf testing as a useful tool for diagnostic and advisory purposes. In particular, it has been noted that low leaf N values could be a result of moisture stress rather than a nutrient deficiency *per se* (Gosnell and Long, 1971).

This paper reviews the Australian and international experiences relating to foliar testing in sugarcane production, and reports on investigations aimed at improving the interpretative skills associated with leaf analysis.

Procedure

Data and information relating to the use of leaf analysis for diagnostic, advisory and nutrient trend purposes in sugarcane production were obtained from various sources. These included results from field and pot trials conducted in the Australian and South African sugar industries, and from records held by the Bureau of Sugar Experiment Stations (BSES), the South African Sugar Association Experiment Station (SASEX) fertiliser advisory service (FAS), CSR Ltd, and other published sources.

In order to improve the interpretive skills associated with leaf analysis, a replicated trial using large pots was conducted to determine the effect of moisture stress on the nutrient content of sugarcane. Sugarcane variety NCo376 was grown in an adequately fertilised sandy loam in 80 litre bins under an automatic rain-shelter for about four months. Moisture stress was applied by withholding water once the cane reached three months of age. Plant growth was measured on an hourly basis using electronic growth transducers linked to a data logger. The young plants (three per bin) were serially harvested at weekly intervals. Total above ground yields were determined for each bin prior to partitioning of the plants into samples corresponding to the spindle and different leaf numbers. The separate components were dried and weighed prior to chemical analysis in the laboratory.

Results and discussion

Critical values

A range of leaf nutrient values (marginal, critical, and/or those considered adequate for cane production) has been established for diagnostic purposes in various sugarcane producing countries (Reuter and Robinson, 1997). For the sake of brevity, only the critical third leaf nutrients values from four world sugar industries are presented in Table 1. These cover the full range of macro and secondary nutrients and some micronutrients or trace elements. Although the nutrient values are not always totally consistent with each other, they most often relate to the middle 300 mm section of the lamina associated with the top visible dewlap (TVD) of the sugarcane plant, which usually corresponds to the third leaf below the spindle (Clements and Ghotb, 1968). Some differences exist in terms of recommended sampling growth stages. The critical values that have been established in the Australian and Mauritian sugar industries refer to samples that need to be collected when the cane is 3–4 and 5 months old respectively. In South Africa and Guyana, the period for sampling has been extended by establishing modified critical values based on crop age. This allows recognition of the fact that N values, in particular, decline with age and time of season. Recognition of varietal differences has also resulted in some 'fine-tuning' of critical values in the South African industry.

Leaf analysis in combination with soil testing is a very useful method for determining balanced nutritional programs for sugarcane. While soil analysis procedures estimate the amount of plant available nutrients, leaf analysis reflects the actual plant nutrient uptake until the sampling date (Smith and Loneragan, 1997). In relation to some micro-nutrients in particular, leaf analysis offers a strong alternative to soil testing in determining the nutrient status of a crop. For example, in the Australian sugar industry, it has been found that the third leaf critical value for zinc is a more reliable index for determining deficiencies than any of the three soil test procedures that were tested (Reghenzani, 1990).

Interpretation of leaf analysis data

As a number of factors influence leaf nutrient content, it is important that guidelines are in place to ensure uniformity in sampling procedure (Appendix 1), sampling season and age of cane. As such, a number of sampling prerequisites exist to enable meaningful interpretation of leaf analysis data. It is commonly accepted that sampling should occur during active growth. Although Evans (1965) suggested that stalk elongation should be greater or equal to 20 mm/day, active growth will normally occur during the months of November to April in Queensland and

Table 1—Critical third leaf nutrient values for sugarcane.

	Critical third (or top visible dewlap) leaf values (%) macro and secondary nutrients; (mg/kg)Cu, Zn, Mn, B, Mo.									
	Australia[a]	South Africa[b]					Mauritius[c]	Guyana[d]		
N	1.8 (3 mnths)	Area	Crop age (mnths)	Month of sampling	P[e]	R[f]	1.95 (5 mnths)	Crop age (mnths)	P[e]	R[f]
		North	3 – 5	Oct–Dec Jan–Feb Mar–Apr	1.9 1.8 1.7	1.8 1.7 1.6		2 3 4.5 5 6	2.4 – 2.5 2.1 1.9	2.4 – 2.5 2.1 1.9
		Coastal	4 – 7	Nov–Dec Jan–Feb Mar	1.9 1.8 1.7	1.8 1.7 1.6				
		Midlands	4 – 9	Nov–Dec Jan–Feb Mar	1.9 1.8 1.7	1.8 1.7 1.6				
P	0.19 (3–4 mnths)	Variety		Areas & crop ages as shown for N			0.21 (5 mnths, ratoon)	Crop age (mnths)	P	R
		N12 Other N & NCo varieties		0.16 0.19				2 3 4.5 6	0.21 0.18	0.21 0.18
K	1.1 (3–4 mnths)	Variety	Harvest season	Month of sampling	Areas & crop ages as shown for N		1.25 (5 mnths, ratoon)	Crop age (mnths)	P	R
		N14	Winter (irrigated crop)	Oct–Nov Dec–Jan Feb–Apr	0.70 0.80 0.90			3–6 2–4.5	1.25	1.25
			Other	Oct–Apr	0.90					
		All other N & NCo varieties	Winter (irrigated crop)	Oct–Nov Dec–Jan Feb–Apr	0.85 0.95 1.05					
			Other		1.05					
Ca	0.2 (3–4 mnths)	0.15 [g]					0.20 (5 mnths)	0.13–0.15 (3 mnths)		
Mg	0.08 (3–4 mnths)	0.08 [g]					0.10 (5 mnths)	0.08 [h]		
S	0.13 (3mnths) S low if N:S>17	0.12 [g]					–	–		
Cu	2 (3–4 mnths)	3 [g]					5 (5 mnths)	3.5 [h]		
Zn	10 (3–4 mnths)	15 [g]					20 (5 mnths)	15 [h]		
Mn	15 (3–4 mnths)	15 [g]					15 (5 mnths)	15 [h]		
B	1 (3–4 mnths)	1 [g]					1 (5 mnths)	1 [h]		
Mo	0.08 (3–4 mnths)	–					0.1 (5 mnths)	0.08 [h]		
Si	0.7 (3–4 mnths)	–					0.7 (5 mnths)	–		

[a] Calcino,1994; [b] Schroeder *et al.*,1992 or Meyer *et al.*,1971; [c] Bassereau, 1988 or Halais, 1962; [d] Evans, 1965.
[e] Plant or replant cane; [f] Ratoon cane; [g] Areas & crop ages as shown for N(S. Afr.); [h] During 'rapid' growth.

December to March in New South Wales, provided that enough well distributed rain fell in the month prior to sampling. Apart from the seasonal consideration, sampling should also take place when the cane is at a satisfactory age. Based on experiences elsewhere (Table 1), it is suggested that sampling ages of 3–5 and 4–7 months be used in Queensland and New South Wales, respectively. In addition, when leaf sampling sugarcane, a period of at least six weeks should have passed since any fertiliser application.

Variety

Variety is another factor that appears to affect nutrient uptake and consequently leaf nutrient values. It has been reported from South Africa that the P and K critical values for sugarcane varieties N12 and N14 respectively (Table 1) are somewhat lower than those associated with the other N and NCo varieties (Schroeder *et al.*, 1993). The CSR leaf testing system that was operative in the Australian sugar industry during the 1960s and 1970s also recognised considerable differences in critical values (referred to as optimum nutrient indices) associated with Queensland varieties for N and K expressed as % dry matter, and P as the P:N ratio, using top visible dewlap leaves (Farquhar, 1965). The optimum nutrient index for Q57 was reported to be 95% of that of Pindar for N, 110% for P and 105% for K. A substantial range in leaf N values have also been reported for varieties grown in Mauritius (Bassereau, 1988).

Diagnosis and Recommendation Integrated System (DRIS) indices

In the development of DRIS, Beaufils (1973), questioned the use of single-valued critical values as he considered the optimum concentration of a particular nutrient to be dependent on the concentrations of other nutrients (Bassereau, 1988). Evaluations of the use of DRIS for sugarcane have indicated that the calculated indices, based on leaf nutrient ratios, are probably more efficient in detecting nutrient imbalances and deficiencies than conventional critical values in young cane (Meyer, 1981; Ng Kee Kwong and Deville, 1983). Despite this, the system is not widely used, as the nutrient index sensitivity appears to diminish with crop age, and there is a general absence of information relating to rates of supplementary nutrient applications based on the DRIS indices.

Moisture stress

It is widely accepted that moisture stress affects leaf nutrient values and restricts interpretation of data (Evans, 1965; Gosnell and Long, 1971; Schroeder *et al.*, 1992). As a result, sampling guidelines emphasise the need to collect samples only when the crop has received enough well distributed rainfall and/or irrigation to preclude any moisture stress. In particular it has been noted, in South Africa, that the analyses of third leaf samples taken from adequately fertilised cane showed low N, P and K values when sampling was conducted during periods of moisture stress (Table 2). However, these values improved substantially when the same blocks were sampled two months later after a number of good rainfall events.

Results from the moisture stress bin experiment showed that 'typical' daily growth patterns occurred in unstressed treatments with a maximum plant extension rate of 2.5 mm/hr being measured in the late afternoon, with daily growth exceeding 25 mm/day. Growth was severely affected during moisture stress, but increased

Table 2—Leaf nutrient values of samples collected from commercial cane in February and April, 1990 (Schroeder *et al.*, 1993)

Field	Variety	Ratoon	Age (mnths)	N (%)	P (%)	K (%)
Sampling date: 20/2/90 (moisture stressed)						
48	NCo376	5	5	** 1.48	***0.12	* 0.90
49	Mixed	5	5	** 1.44	***0.14	** 0.83
50	NCo376	7	5	***1.30	***0.12	** 0.83
66	Mixed	2	4	***1.32	***0.14	1.31
Sampling date: 24/4/90 (stress relieved)						
48	NCo376	5	7	1.71	0.18	1.46
49	Mixed	5	7	1.87	0.19	1.32
50	NCo376	7	6	1.61	0.19	1.43
66	Mixed	2	6	1.76	0.20	1.52
* Marginal, ** Low, *** Very low						

substantially once moisture stress was relieved. The same trend occurred in the leaf N values (Figure 1), and was also reflected in the measured leaf moisture potential and photosynthetic rate values (not presented here).

Although, in this instance, moisture stress has been singled out as an important factor that influences leaf nutrient values, cognisance should always be taken of other factors, such as pests, disease, etc., which may contribute towards non-representative leaf nutrient values. Cane affected by such conditions should always be avoided during sampling.

Leaf analysis for advisory purposes

Apart from the diagnostic role associated with leaf analysis, a number of attempts have been made to use foliar analysis data, either solely or partially, for calculating crop nutrient requirements. These include the so-called 'Clements crop log system' as devised and developed in Hawaii, the CSR leaf analysis service and the SASEX concept of 'whole cycle fertiliser advice'.

Crop logging

The sugarcane crop log system (Clements, 1959), that was established in the 1940s, is aimed at providing a means of detecting and correcting any nutrient (and/or water) deficiencies with minimum delay (Whalley and Clarkson, 1950). With this system, samples (leaves 3–6 for N analysis and their sheaths for fresh weight, moisture, total sugars, P, K, Ca and Mg) are collected every 35 days. The resulting nutrient indices (N, Ca and Mg as a percentage of dry matter, and P and K as percentages of sugar-free dry matter) are recorded and charted throughout the life of the crop (Bassereau, 1988) and compared with desirable index values. Fertiliser additions would be made to alleviate any deficiencies that were identified as the crop progressed. Despite wide use in Hawaii in earlier years, crop logging has

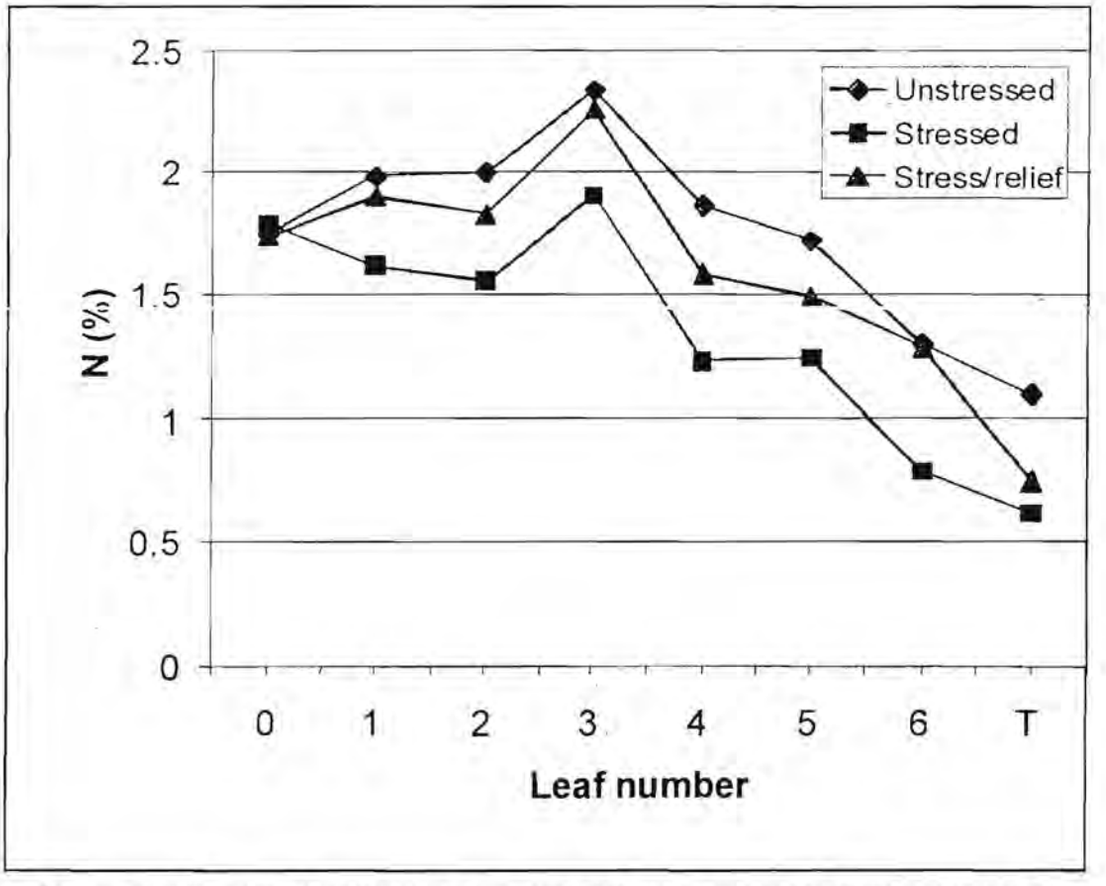

Fig. 1—Leaf N (%) values of the partitioned plants associated with no moisture stress, moisture stress and stress/relief conditions. (T = trash)

not been widely accepted, probably as a result of the very intense sampling program associated with the system.

CSR

In Australia, the CSR leaf testing service provided fertiliser recommendations for growers supplying cane to their mills, by using optimum leaf nutrient indices and a number of nutrient action levels (range of values above and below the optimum nutrient indices). These were updated on an annual basis from the results of NPK factorial trials in each mill area. Analysis data of leaf samples from individual blocks were compared to the appropriate action levels (based on variety) to determine appropriate adjustments to previous fertiliser application rates. For instance, the 1973 N advice for the Herbert River district (Table 3) was based on the action levels that had been compiled from the results of the 1972 trials. A third leaf analysis value of 2.15% for a particular block of cane would have resulted in no change to the N fertiliser program (as used for the previous crop) if the variety were Triton. However for variety Q68 and Pindar, the recommendation would have been respectively 52 kg/ha less and 52 kg/ha more than the previous application rates.

This service was withdrawn during the mid-1970s due to an apparent lack of support by the grower community and as a result of resources being required elsewhere.

SASEX whole cycle fertiliser advice

The primary objective of the SASEX fertiliser advisory service is to provide growers with fertiliser recommendations for a cycle of plant (or replant) crop and four successive ratoons. Such 'whole cycle' fertiliser advice is based on the analysis of soil samples collected prior to planting. Leaf analysis, particularly taken during the ratoon crops, is used to check on the adequacy of the original recommendations (Schroeder *et al.*, 1993). Although the system is essentially geared for confirmation or correction of the fertiliser program for the subsequent crop, guidelines for additional fertiliser applications to the current crop are available for N, P and K (Table 4). The proviso exists that the cane should be young enough (3–5 months of age) to enable effective crop utilisation of any supplementary nutrient dressings.

Conclusions

The evidence presented in this paper suggests that leaf analysis is a useful diagnostic and advisory 'tool' that has not yet been fully utilised in the Australian sugar industry. Attempts by CSR, in the past, to provide fertiliser recommendations based on leaf analysis apparently failed because it was used in isolation from other diagnostic 'tools' such as soil testing. Apart from the recognition of varietal differences, it appears that not enough consideration was given to the other factors that influence leaf nutrient values such as time of sampling, crop age, moisture stress, etc.

It is now widely accepted that the most reliable assessment of the nutrient requirement for sustainable production in a particular block of sugarcane will undoubtedly be obtained from the use of a combination of the various 'tools' available to the grower and/or his adviser. Apart from its popular use for diagnosing nutrient deficiencies, leaf analysis has been successfully used in certain other sugar industries to amended fertiliser programs for the following season. However, if the cane is sampled early enough, it may also be used to determine supplementary fertiliser applications for the existing crop. Leaf analysis has also been found to be a useful means of conducting nutrient surveys at regional and industry levels to identify nutrient trends and/or potential imbalances. At the farm scale, leaf analysis offers a very useful way of ensuring

Table 3—CSR nitrogen action levels—Herbert River, 1972 (CSR unpublished data—Report on technical field work 1972–1973)

Sugarcane variety			Recommendation
Pindar	Triton	Q68	
Leaf N (%)			
> 2.49	> 2.30	> 2.29	Previous N rate – 103 kg/ha
2.35 – 2.49	2.16 – 2.30	2.15 – 2.29	Previous N rate – 52 kg/ha
2.20 – 2.34	2.01 – 2.15	2.00 – 2.14	Previous N rate + 0
2.05 – 2.19	1.86 – 2.00	1.85 – 1.99	Previous N rate + 52 kg/ha
1.90 – 2.04	1.71 – 1.85	1.70 – 1.84	Previous N rate + 103 kg/ha
< 1.90	< 1.71	< 1.70	Investigate

Table 4—Recommendations for additional N, P and K application based on leaf analysis data in the South African sugar industry (Anon., 1996).

	Leaf nutrient value (%)			
	Nitrogen			
Leaf N (%)	< CV–0.4	CV–0.2 to CV–0.4	CV to CV–0.2	> CV
N required (kg/ha)	100	75	50	0
	Phosphorus			
Leaf P (%)	< CV–0.03	CV–0.03 to CV–0.02	CV–0.01 to CV	> CV
P required (kg/ha) Weakly P sorbing soils Strongly P sorbing soils	30 80	20 50	20 30	0 0
	Potassium			
Leaf K (%)	<CV–0.2	CV–0.2 to CV–0.1	CV–0.1 to CV	>CV
K required (kg/ha)	150	100	50	0

CV = Critical value as indicated in Table 1.
Leaf nutrient value = CV minus a specified value.

balanced nutrition and the prevention of trace element deficiencies in particular.

The fact that general leaf critical values and guidelines for sampling already exist in the Australian sugar industry could enable leaf analysis to be easily incorporated into the local diagnostic and advisory system. Although it is at present recommended that samples be collected within the current guidelines to ensure meaningful interpretation of the analytical data, due consideration should be given to factors such as age of crop at sampling, climatic conditions and soil properties. Current glasshouse investigations could provide valuable data and information for improving the interpretive skills required to assess varietal differences and the effects of moisture stress on nutrient uptake. The use of leaf nutrient ratios could be useful in this regard, particularly in relation to drought-affected cane. The development of a chemical/biochemical indicator of moisture stress and modified sampling strategies, currently under consideration, could have important implications for identifying samples affected by such conditions at the time of collection. Similar investigations relating to waterlogged conditions following above average rainfall could also be rewarding.

Renewed usage of leaf analysis within the Australian sugar industry will not only be important for nutritional research and extension, but will also ensure that growers are able to make more informed decisions regarding nutrient management on the farm and therefore maximise their own potential economic returns.

Acknowledgments

The experimental work associated with the effect of moisture stress on the nutrient content of sugarcane was conducted while the senior author was employed by the South African Sugar Association Experiment Station (SASEX), Mt Edgecombe, South Africa, and forms part of his PhD project at the University of Pretoria.

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Appendix 1

Recommended leaf sampling procedure for the Australian sugar industry

- Select leaves from stalks of average height.
- Sample the third leaf from the top of the stalk. The first leaf is the one that is more than half unrolled.
- Collect about 40 such leaves from the block of sugarcane, preferably using a diagonal sampling pattern.
- Fold the leaves in half (tip to base) and cut a 100 mm length from the folded leaf (giving a total of 200 mm).
- Strip out and discard the midrib from the 200 mm section.
- Place the sample in a clean paper bag and keep in a cool environment (polystyrene cooler) until the sample can be dried in an oven, or in a well-ventilated area.
- Once the sample is dry, send it to the laboratory for analysis.
- Supply the following information:
  - Name, address and mill area;
  - Block number;
  - Variety;
  - Crop (plant or ratoon number);
  - Sampling date and age of the cane at harvest;
  - Details of fertiliser applied (type and rate).
- Always ensure:
  - Requirements for sampling in terms of season, age, time lapse from fertiliser application, etc. are met;
  - Hands are clean when sampling;
  - Cane is not affected by some other factor, e.g., disease, insect damage, abnormal climatic factor, etc.;
  - Samples are not contaminated by fertilisers and/or other chemicals.