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

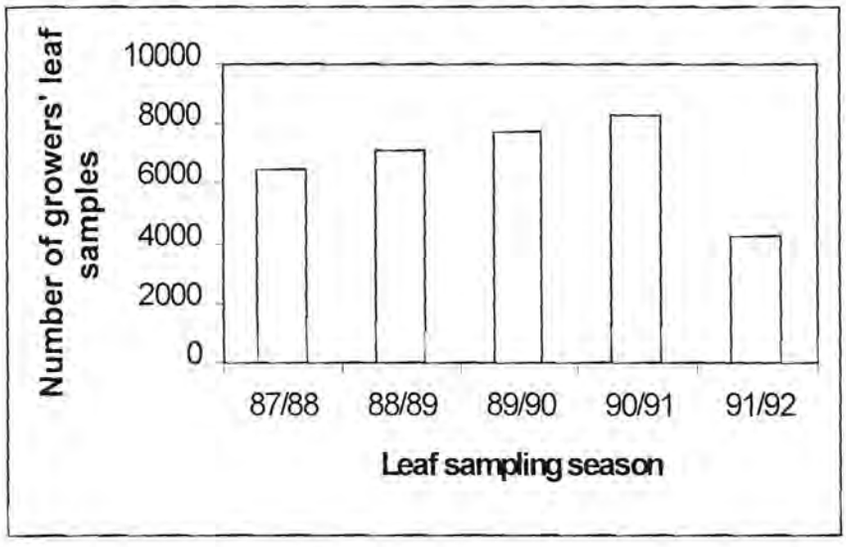


Table 2.1. Mean rainfall figures for the South African sugar industry during the growing seasons 1986/97 to 1990/91.

	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

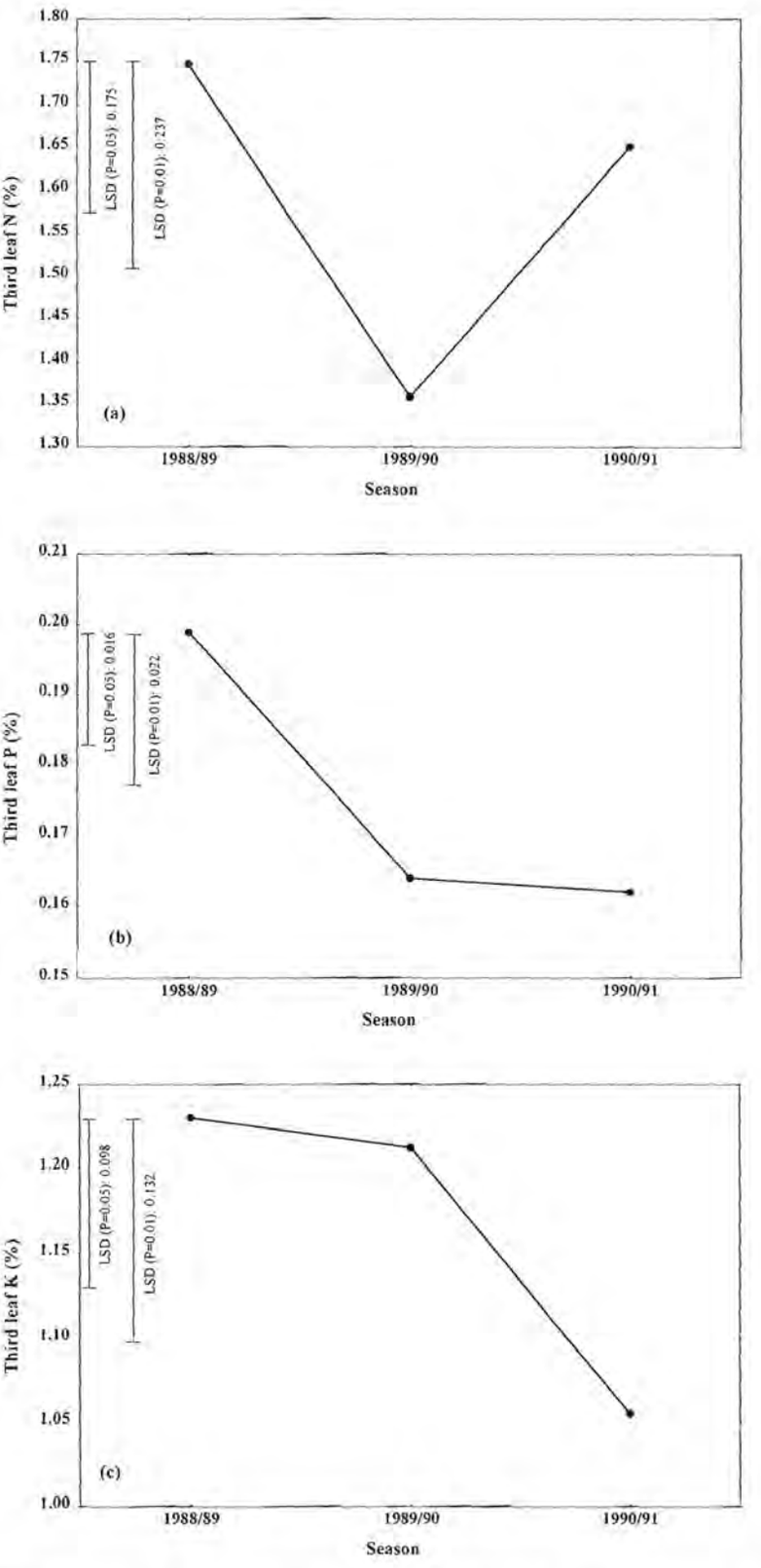


Figure 2.2. Leaf nutrient values associated with the samples collected from the Zululand estate during the 1988/89, 1989/90 and 1990/91 seasons.

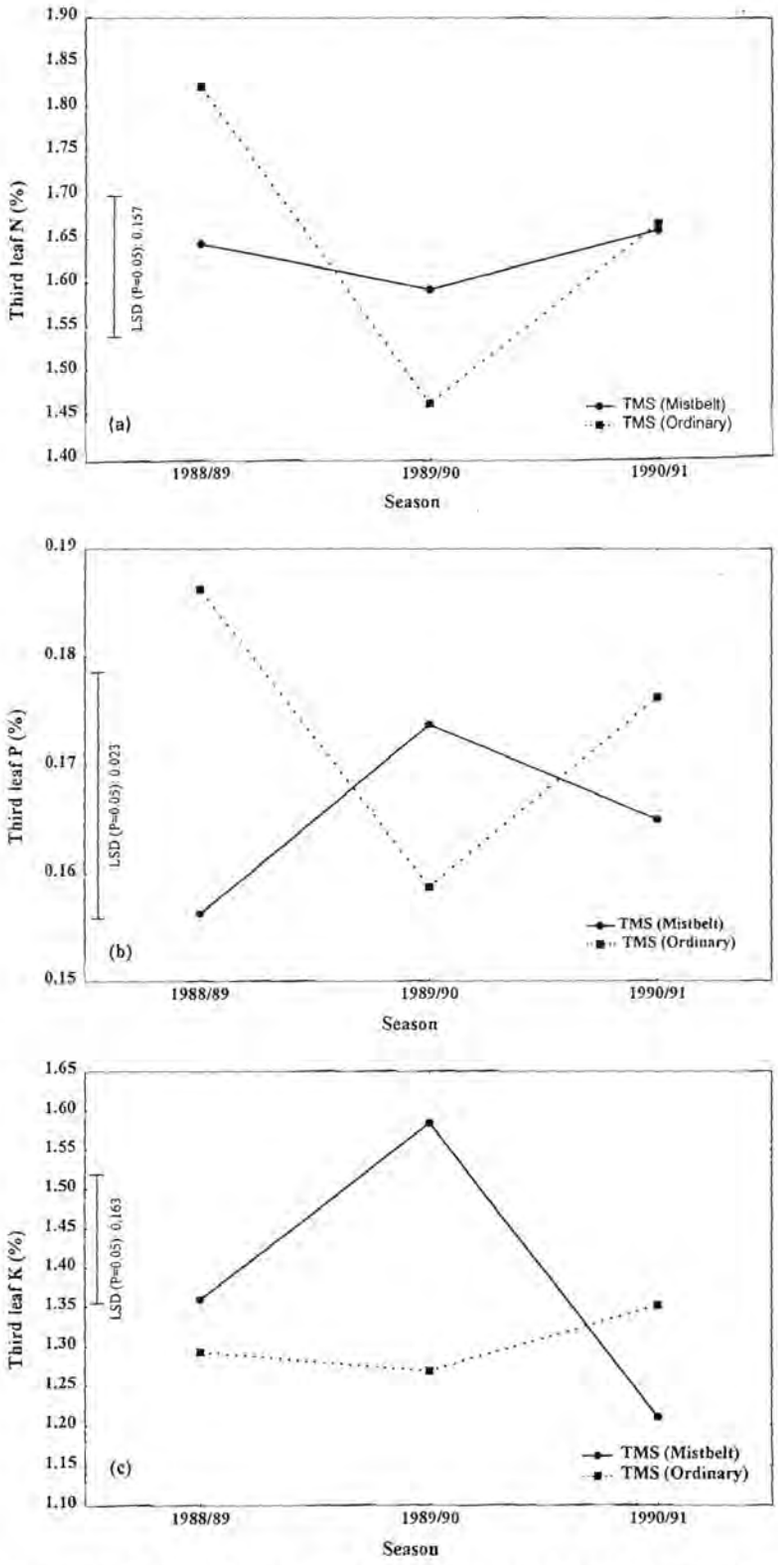


Figure 2.3. Leaf nutrient values associated with the samples collected from the Midlands estate during the 1988/89 (including 1987/1988), 1989/90 and 1990/91 seasons.

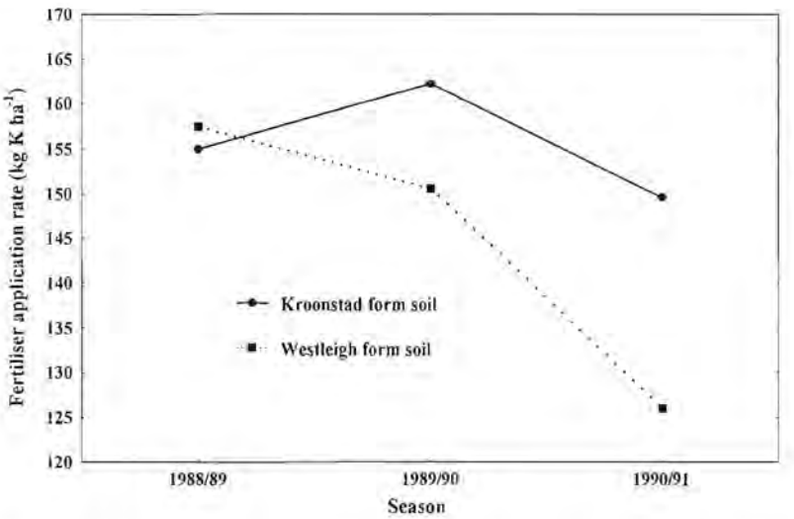


Figure 2.4. Potassium fertiliser application rates on the different soil forms on the Zululand estate over the period 1988/89 to 1990/91.

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.

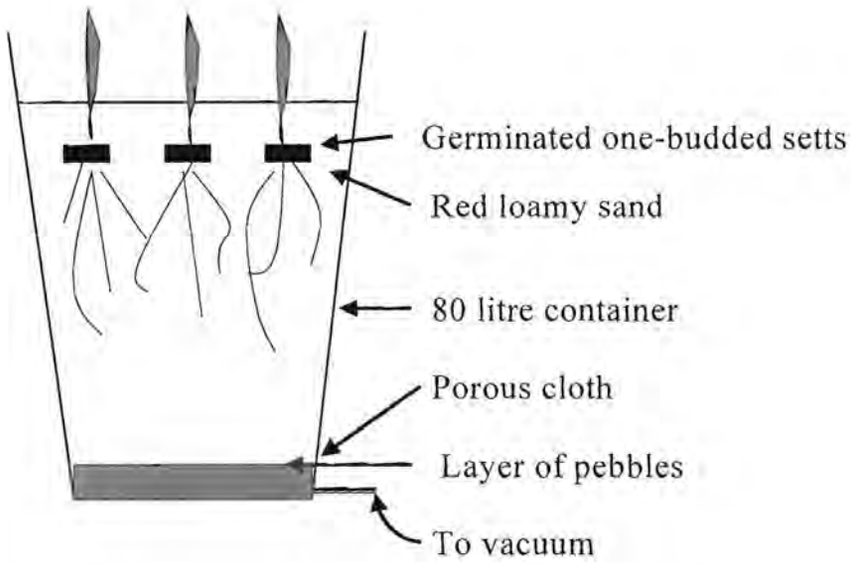


Figure 3.1. A diagrammatic representation of the planted containers used in the investigation.

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.

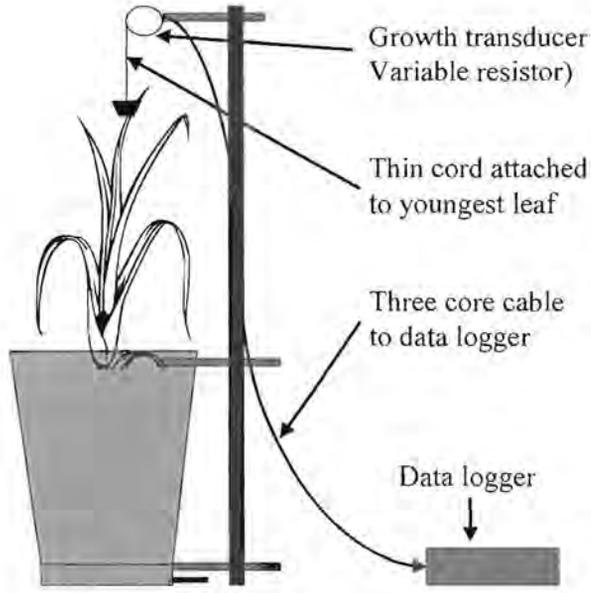


Figure 3.2. Measurement of hourly plant extension rate - diagram showing the connection of a growth transducer to the youngest leaf of one of the plants in a container.

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).

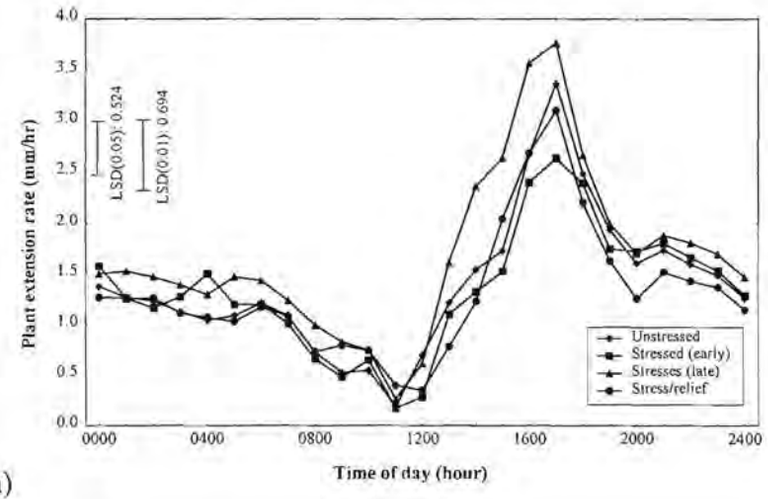
The difference in mean HPER value between the future "stressed (late)" treatment and the other future treatments was not considered a problem, as in all cases the mean daily plant extension rate over a three day period prior to the imposition of the moisture stress treatments (Table 3.2) was above 20mm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

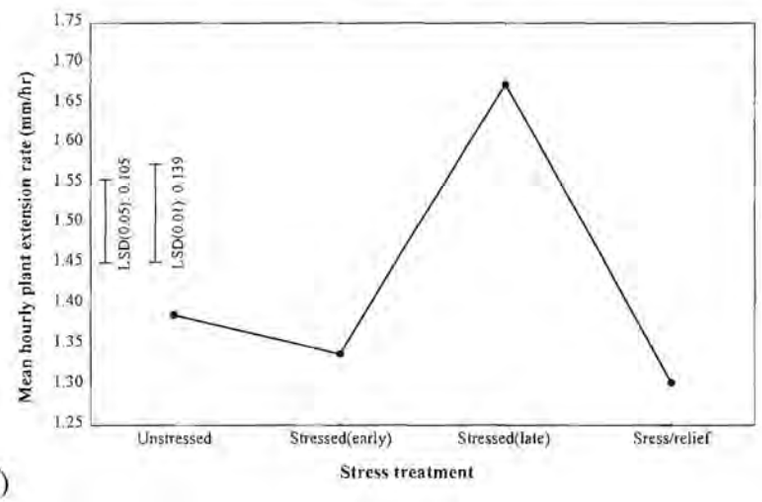
Future moisture stress treatment	Mean DPER (mm day^{-1})
Unstressed	37.0
Stressed (early)	36.5
Stressed (late)	46.4
Stress/relief	38.8
SE	2.1
LSD (0.05)	6.5
LSD (0.01)	9.2

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.

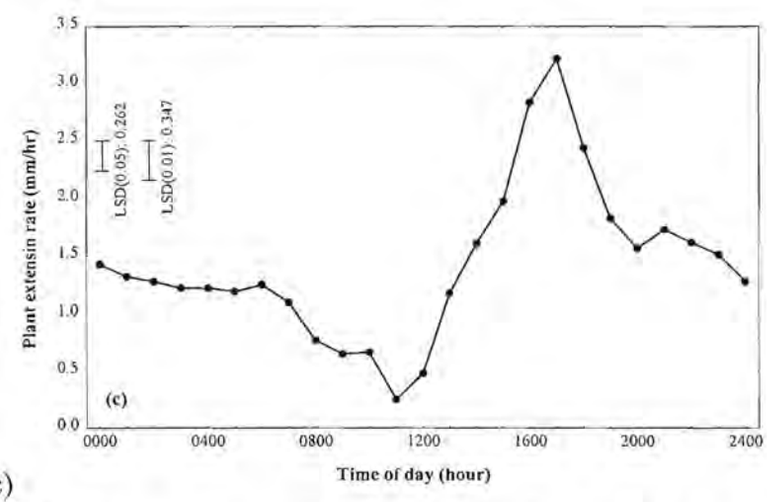
As the soil used in this investigation had a relatively low water holding capacity (12%), moisture stress effects on plant growth soon became visually apparent within the treatments in which irrigation was withheld. These negative responses to stress were reflected in mean DPER values recorded over three-day 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).

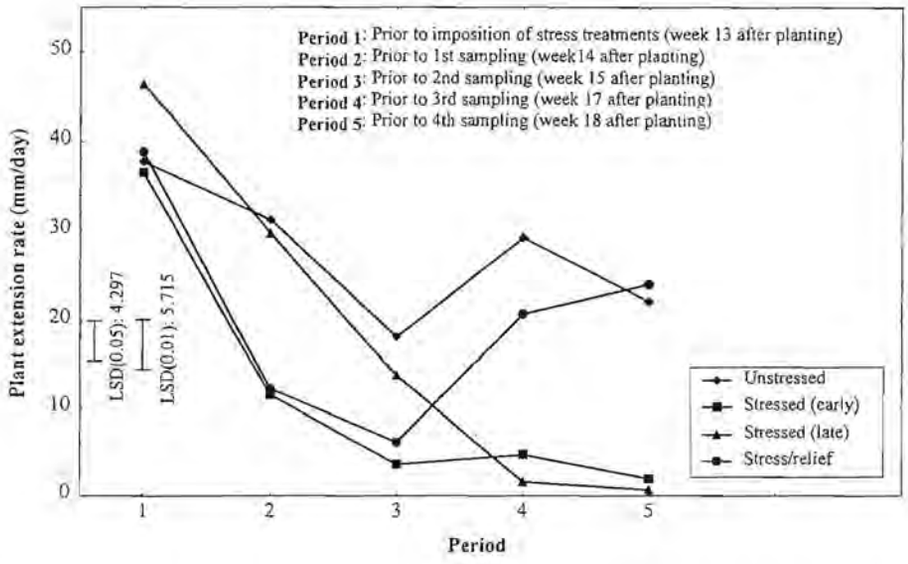


Figure 3.4. Mean plant extension rate values associated with the various moisture stress treatments with time.

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*.

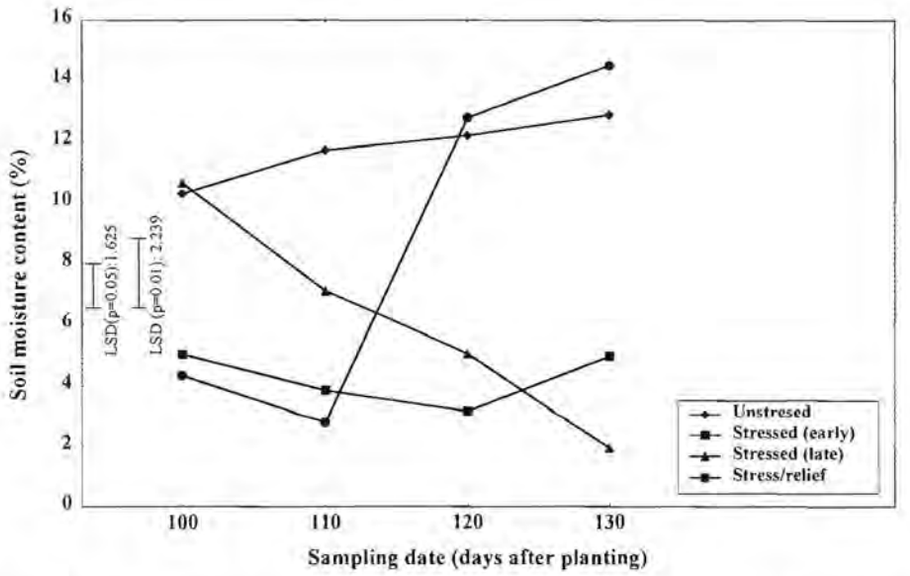


Figure 3.5. Mean soil moisture content values associated with the unstressed, stressed and stress/relief treatments over the thirty-day harvest period of the investigation.

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).

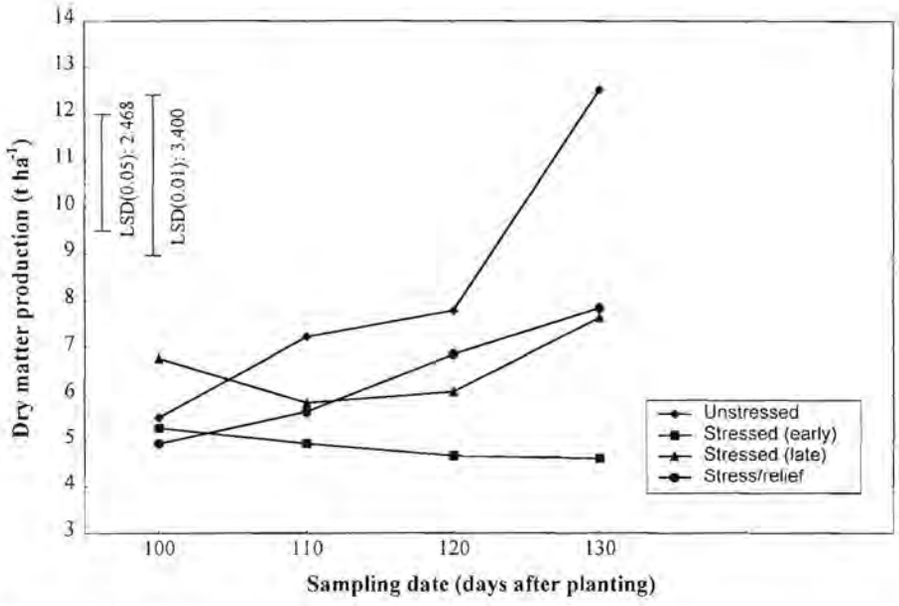


Figure 3.6. Interactive effect between moisture stress and sampling date on dry matter yield.

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.

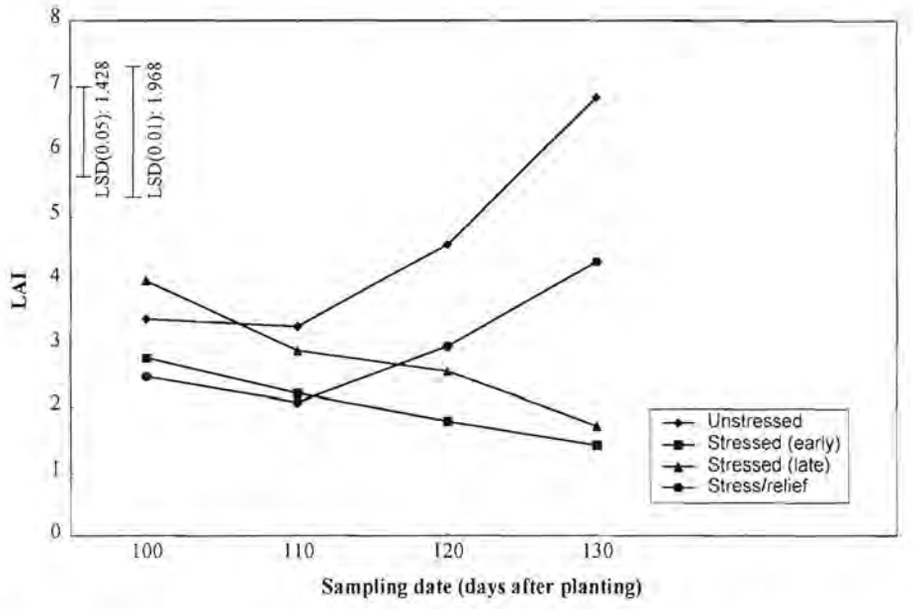


Figure 3.7. Interactive effect between moisture stress and sampling date on LAI.

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

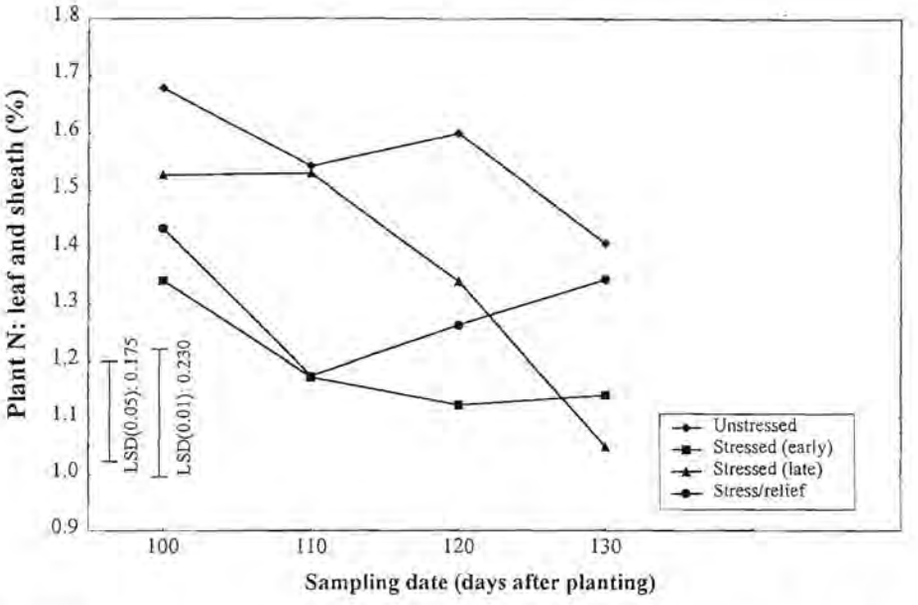


Figure 3.8. Interactive effect of moisture stress and sampling date on plant N content.

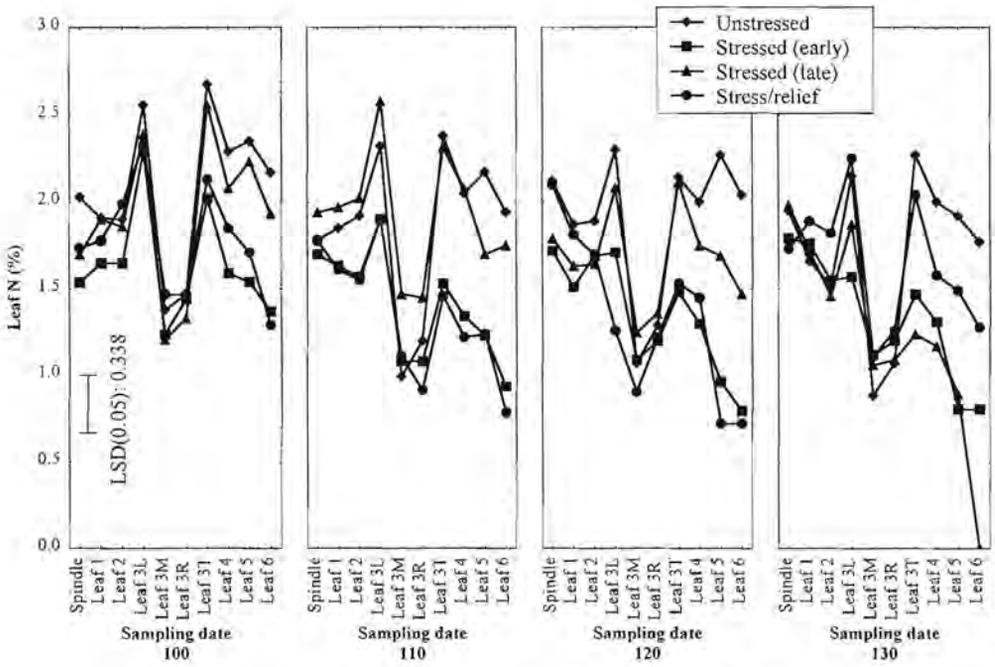
Apart from the interactive effect on the N content of the entire plant, these general moisture stress X sampling date effects were also generally apparent in the various plant components (Figure 3.9).

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.

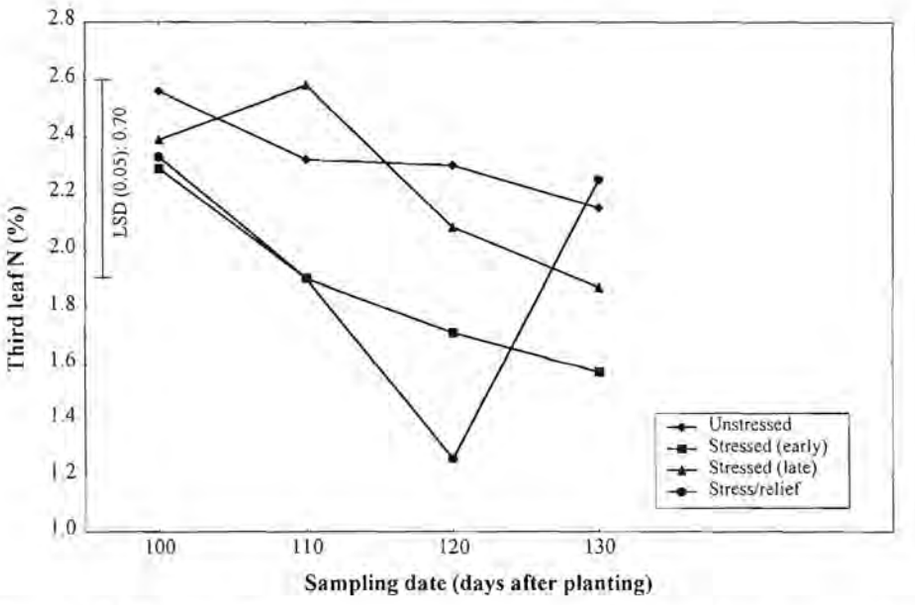


Figure 3.10. The effect of moisture stress and sampling date on third leaf N values.

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

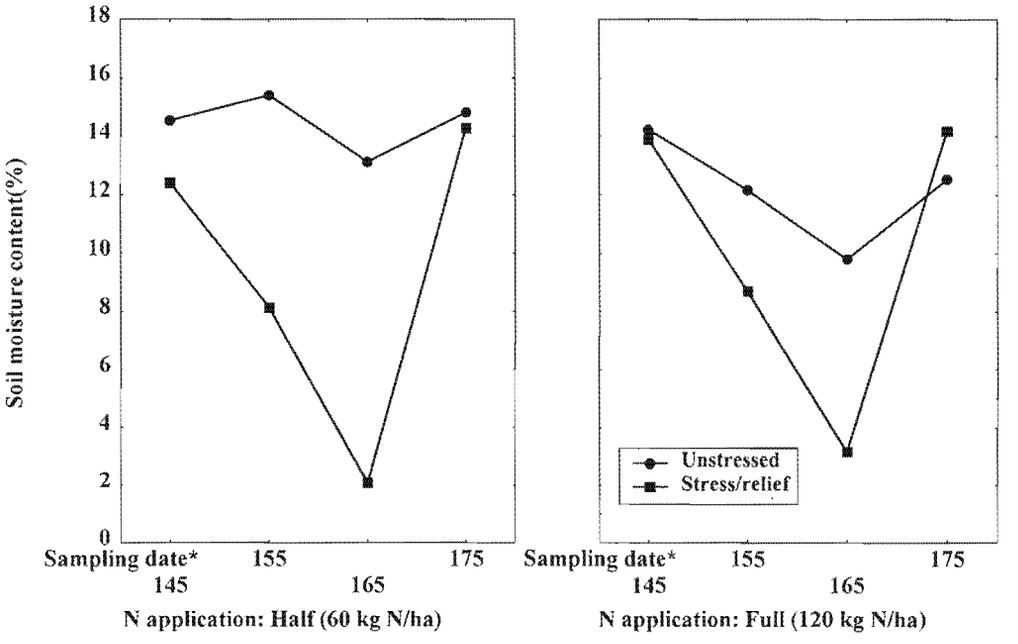
4.3.1. Effect of moisture stress treatments on soil moisture content and LAI

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.

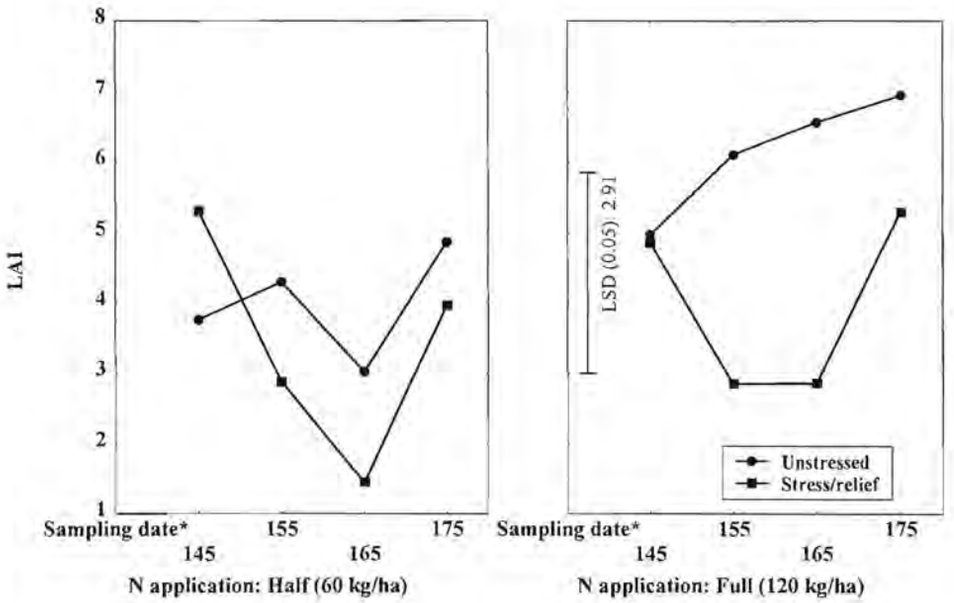


* days after planting

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).

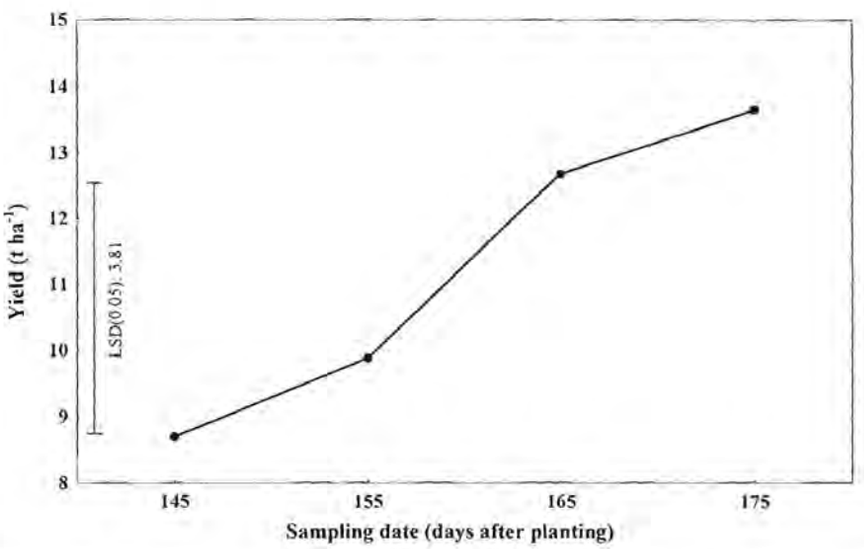


Figure 4.3. Dry matter yield as measured over the 30-day sampling period.

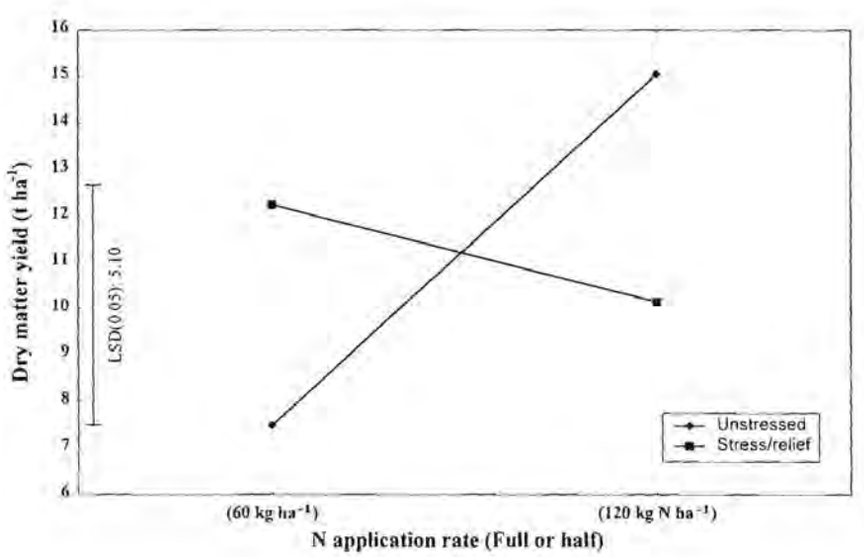


Figure 4.4. The interactive effect of N application rate and moisture stress treatment on dry matter yield.

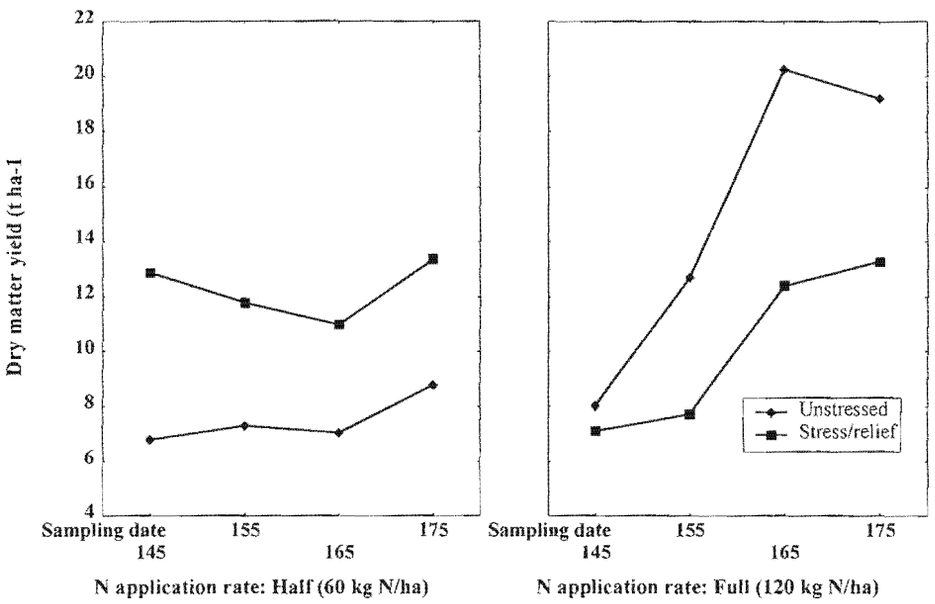


Figure 4.5. The influence of N application rate and moisture stress treatment on dry matter yield over the 30-day sampling period.

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.

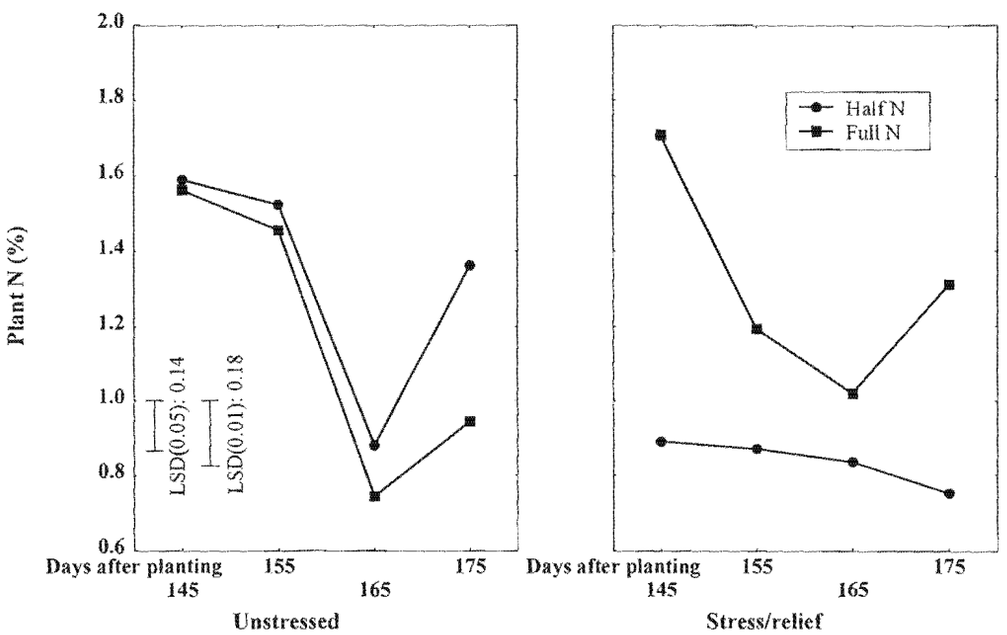
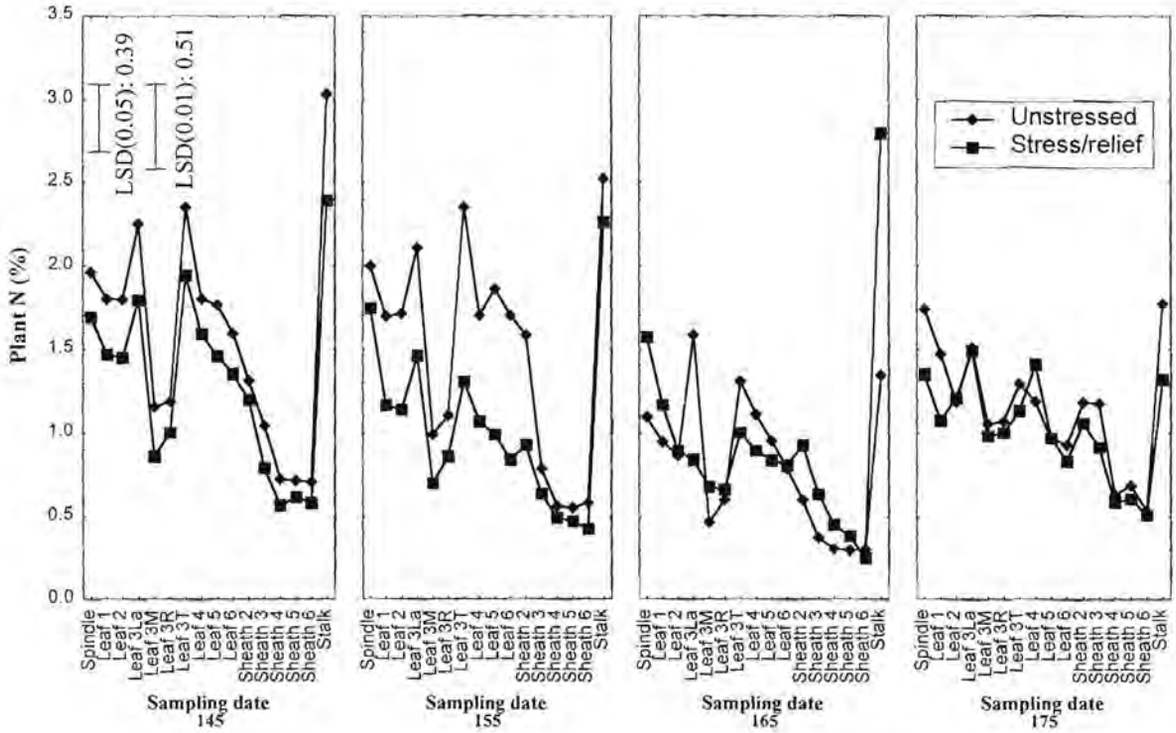


Figure 4.6. Interactive effect of N application rate and sampling date on plant N(%).

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.

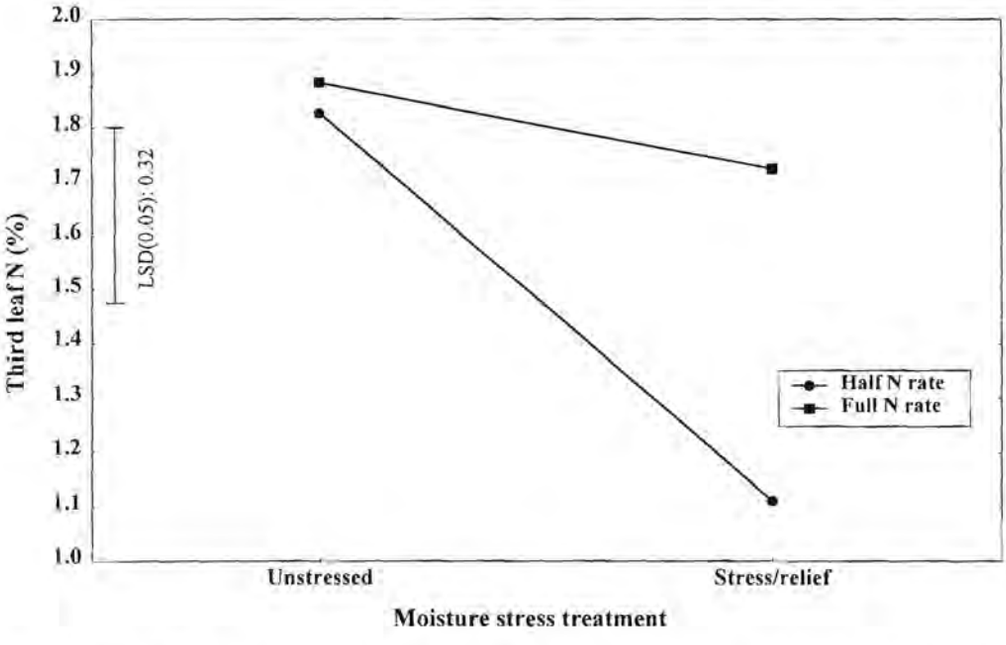


Figure 4.8. The interactive effect of N application rate and moisture stress on third leaf N values.

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.

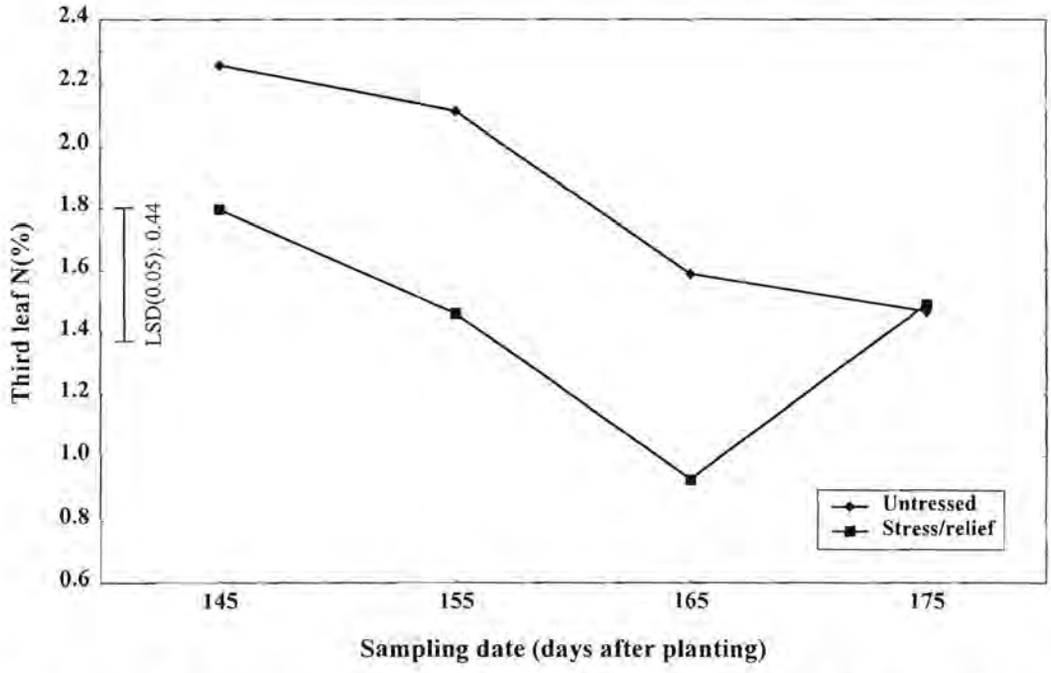


Figure 4.9. The effect of moisture stress and sampling date on third leaf N values

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).

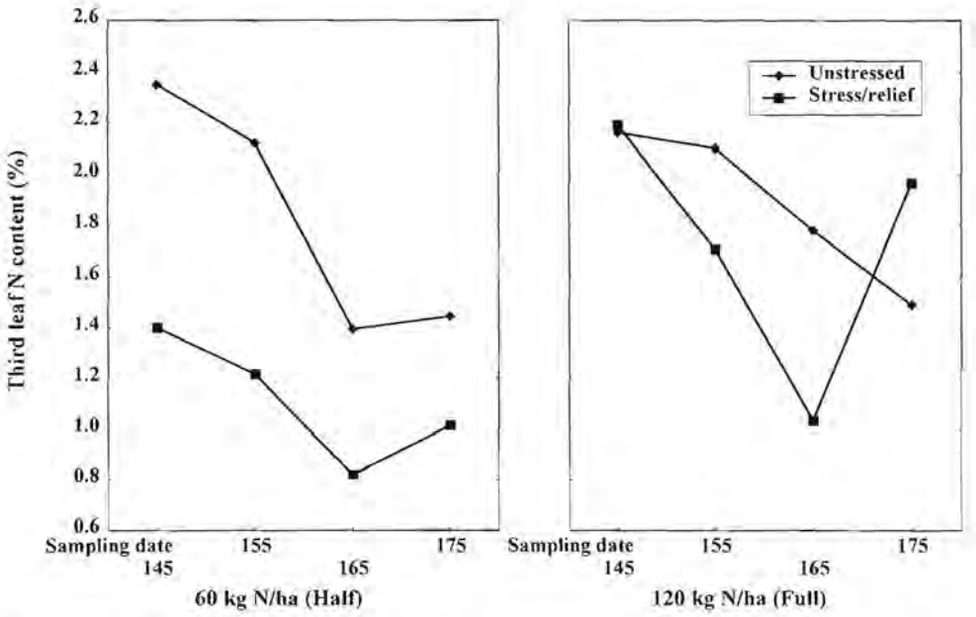


Figure 4.10. The influence of N application rate and moisture stress on third leaf N values.

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*.