Chapter 5.

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

5.1. Introduction

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

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

5.2. Materials and methods

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

5.3. Results and discussion

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

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

The main effects associated with the analysis of variance (four moisture stress treatments, four sampling dates and 15 plant parts) indicated that highly significant differences existed between the mean total plant P (%) values within the various 'treatments'. This related to the moisture stress treatments, sampling date and plant parts (Table 5.1). The total plant P in the unstressed treatment was significantly different ($P<0.01$) from that of the other treatments (stress related), and total P in the stress/relief treatment was significantly different from that of the comparable stressed (early) treatments. This indicated that some recovery in plant P occurred when moisture stress was relieved. As with N, plant P also declined with increasing leaf and sheath number. The P content of the sheaths was generally lower than that of their associated lamina, although the leaf and sheath P values of the second leaf were similar. In relation to the partitioned third leaf, the P content of the mid 200mm section with the midrib removed (L3La) was similar to that of the top section of the leaf (L3T), but significantly higher than that of the midrib (L3M) and the lower section of the leaf (L3R).

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

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

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

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

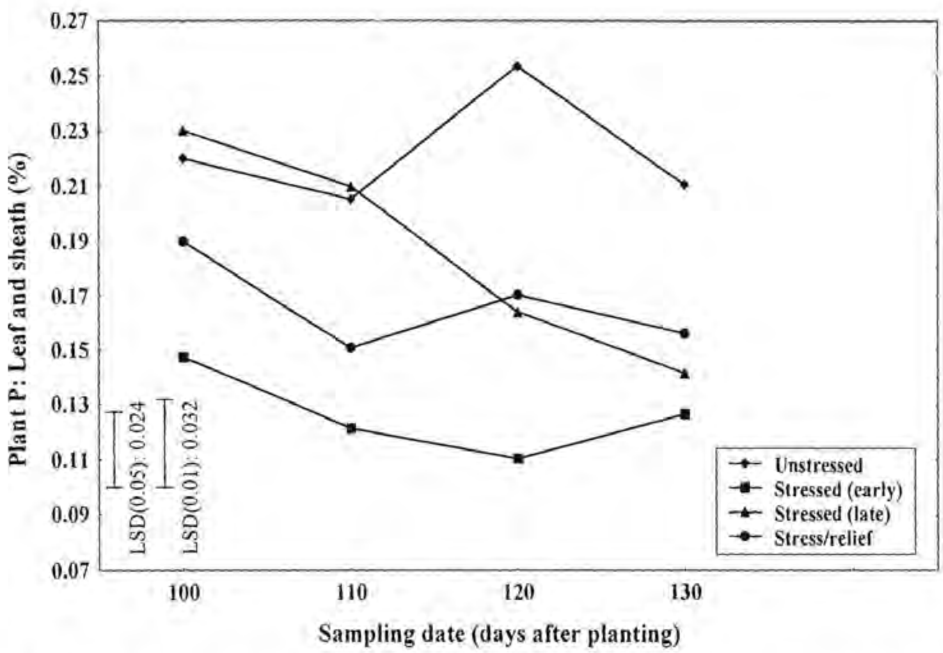
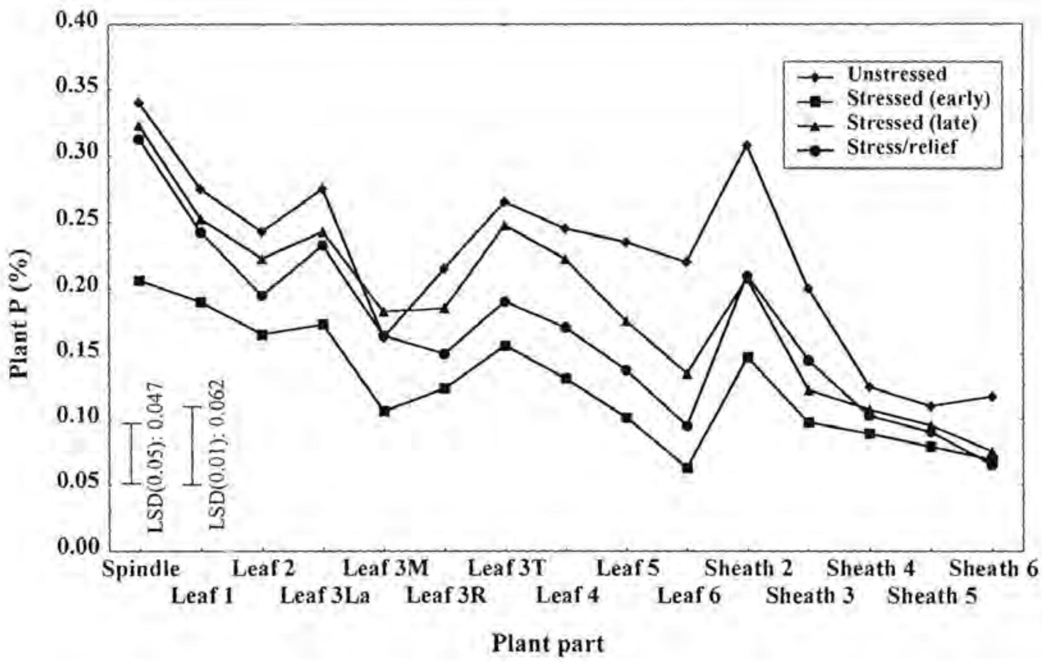


Figure 5.1. The interactive effect of moisture stress and sampling date on total plant P.

In relation to the stress treatment x plant part interaction (Figure 5.2), it was found that mean P values associated with the different plant parts (over the four harvest dates) under conditions of moisture stress (stressed (early)) were generally significantly lower than those of the corresponding unstressed conditions. The exceptions (not significantly different) were those associated with the lower sheaths (sheaths 4, 5 and 6) and the midrib of the third leaves (Leaf 3M). The mean plant P values of the various plant parts associated with the stressed (late) were similar but lower than those associated with the unstressed conditions. In relation to the stress/relief treatment the leaf P values were generally higher (not always significant) than those associated with the stressed (early) treatment. However, this did indicate that some recovery in P values occurred, especially in the actively growing part of the plant (spindle, and lamina and sheath of leaf one).



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

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

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

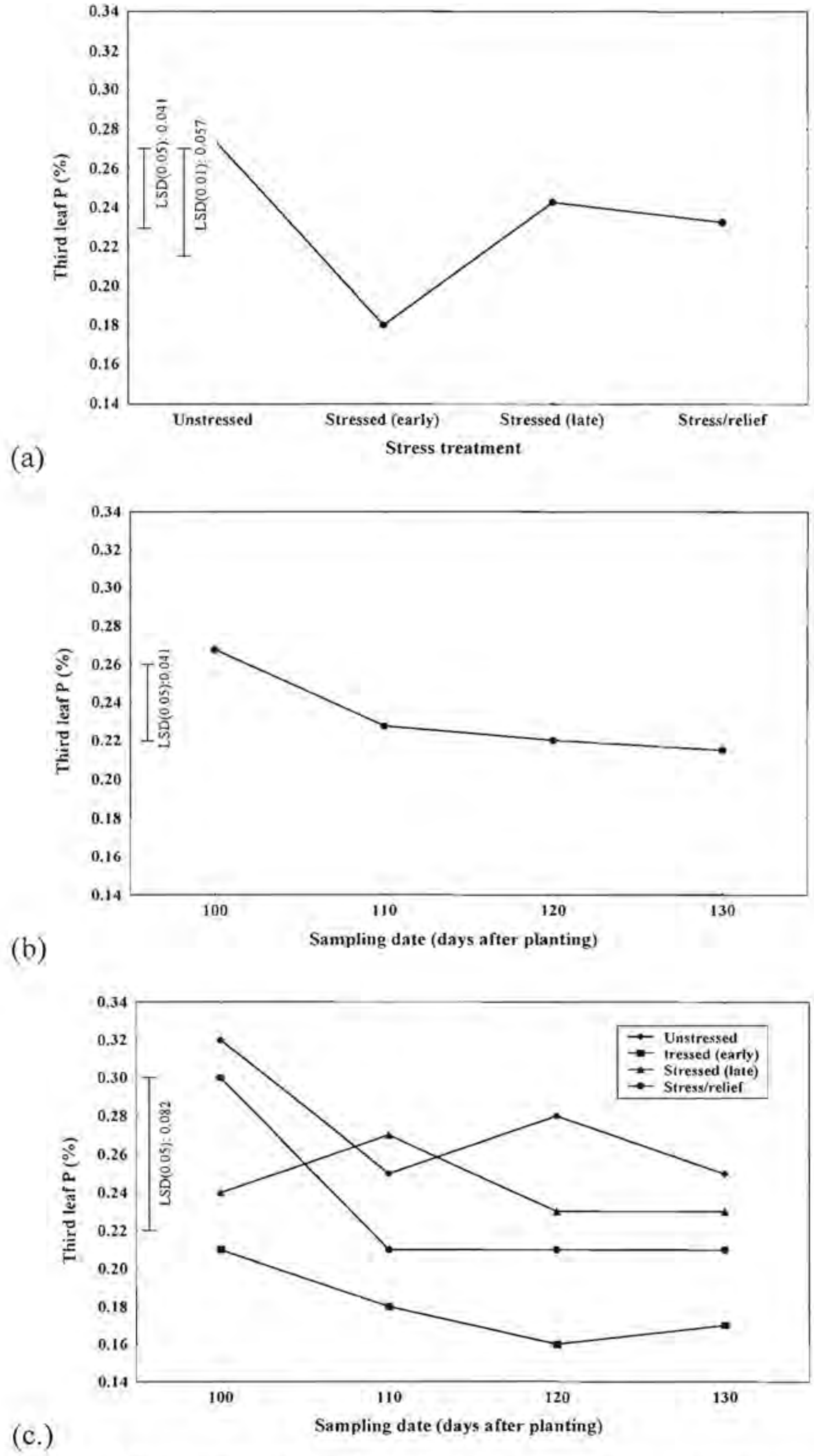


Figure 5.3. Third leaf P (%) as affected by moisture stress treatment (a), sampling date (b) and the interaction between sampling date and moisture stress treatment (c).

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

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

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

The main effects associated with the analysis of variance (two moisture stress treatments, four sampling dates and 16 plant parts) indicated that highly significant differences existed between the mean total plant P (%) values (covering all the plant parts considered) within all the 'treatments' (Table 5.2). The total plant P in the unstressed cane was significantly different (P0.01) from that of the stress/relief treatment. In the case of "sampling date", it was found that the mean plant P values were generally not dissimilar to each other, the exception being the total plant P associated with the third sampling date (165 days after planting and 25 days after water was withheld). This unusual decline and subsequent improvement in mean total plant P was thought to be associated with possible uncharacteristically dry conditions (as described in Chapter 4) that may have affected the cane on the third sampling date. In relation to the plant parts, P declined with increasing leaf and sheath number. With the exception of the second leaf, the P contents of the sheaths were lower than their corresponding laminae. The data relating to the P content of the partitioned third leaf showed similar trends to those identified in Trial 1 (Table 5.1). The lamina (L3La) had a P value significantly higher ($P<0.05$) than that of the midrib (L3M) and lower section of the lamina (L3R), but similar to (not significantly different from) that of the top section of the lamina. The stalk was found to have the highest P content, which was significantly different ($P<0.01$) from all the other plant parts except the spindle.

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

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

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

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

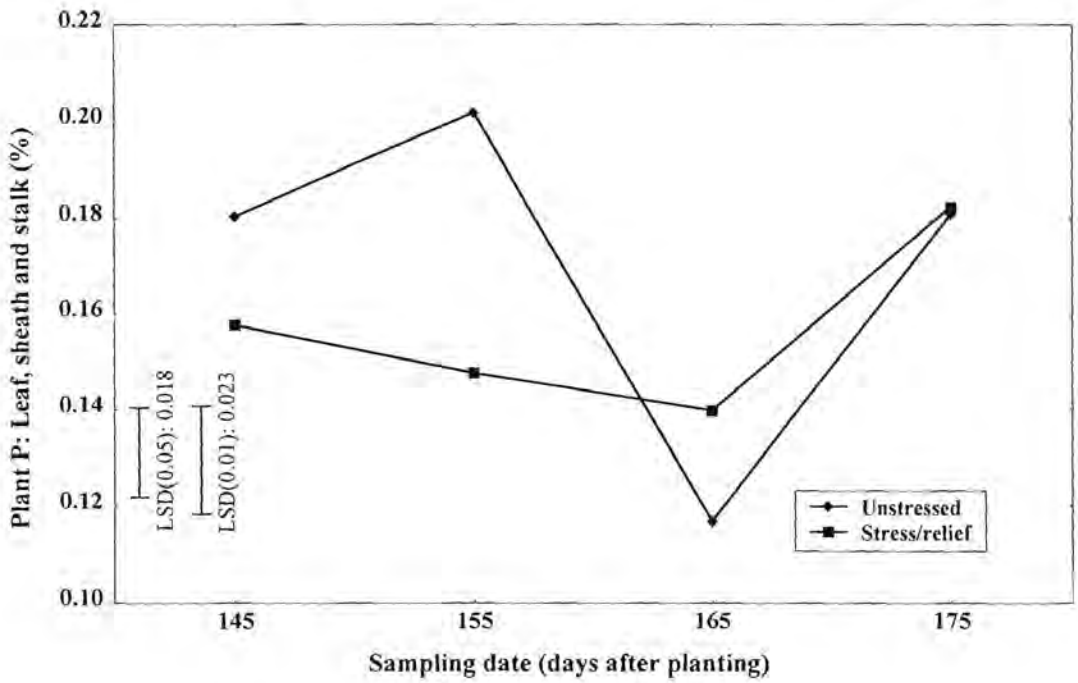


Figure 5.4. The interactive effect of moisture stress and sampling date on total plant P.

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

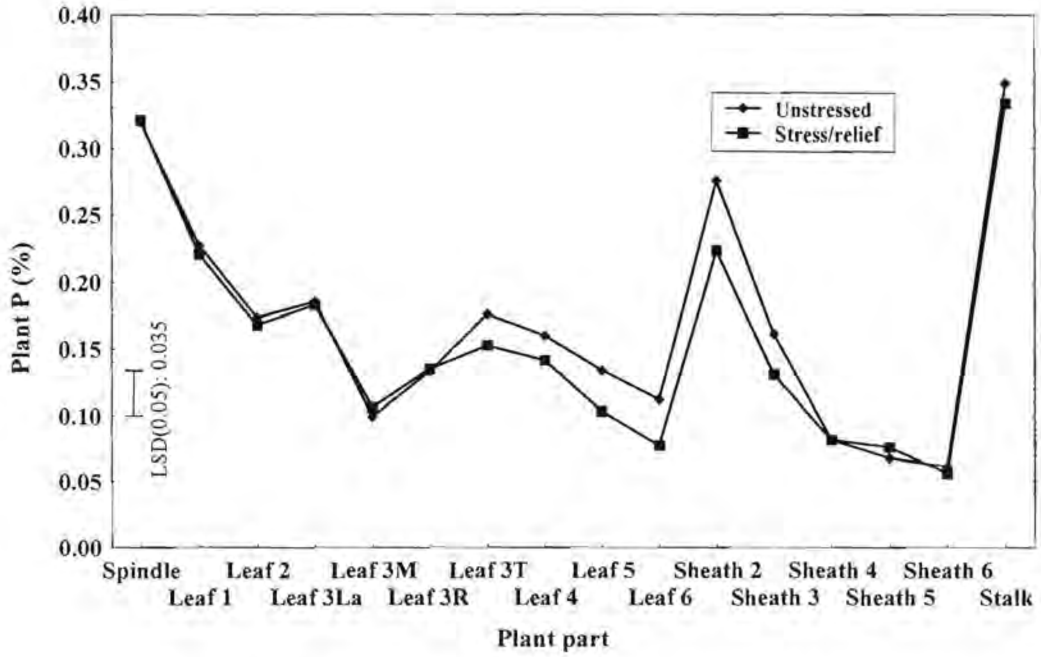


Figure 5.5. Plant P (%) of the different plant parts associated with the two moisture stress treatments.

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

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

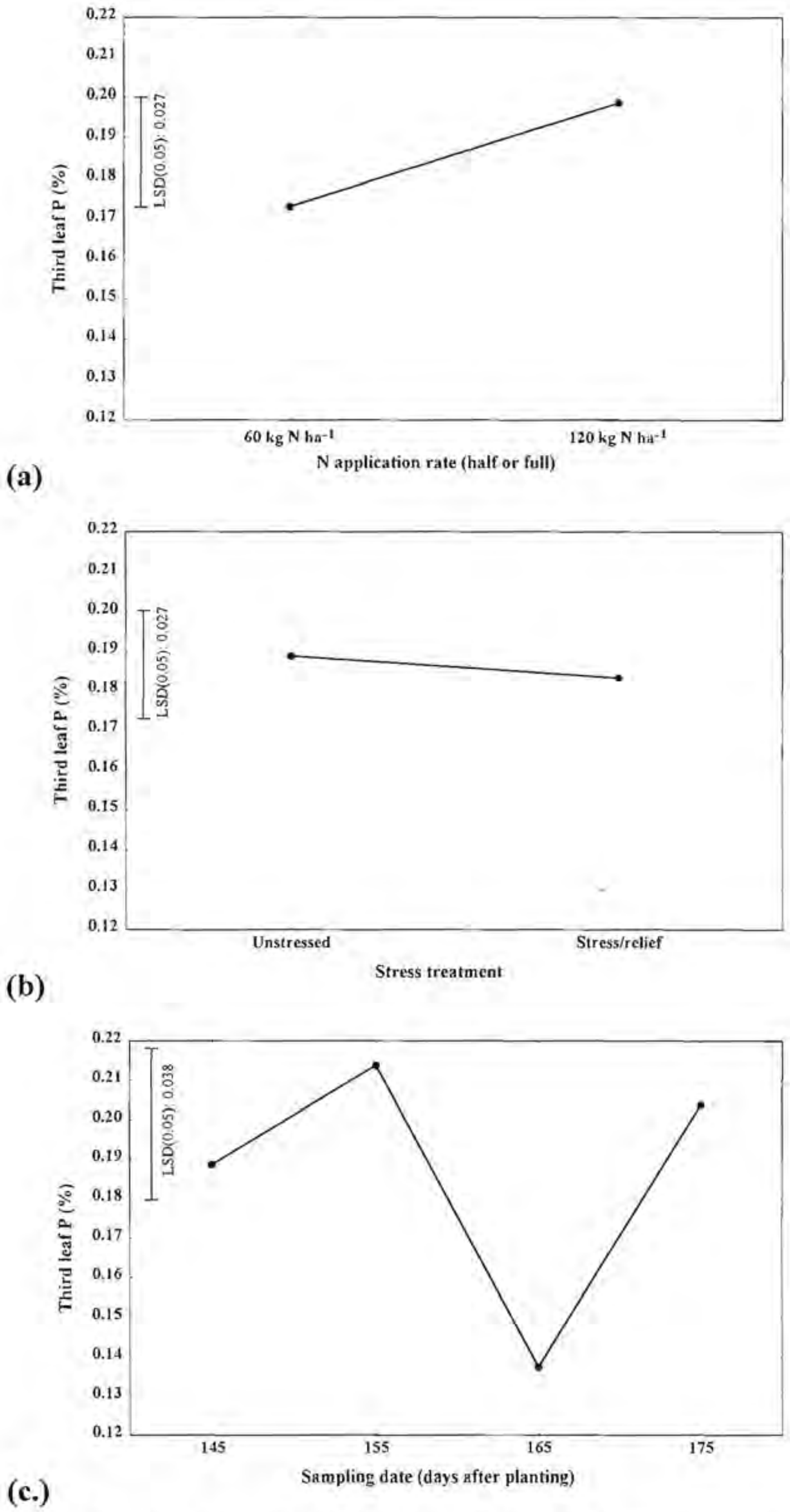


Figure 5.6. Third leaf P (%) as affected by N application rate (a), moisture stress treatment (b) and sampling date (c).

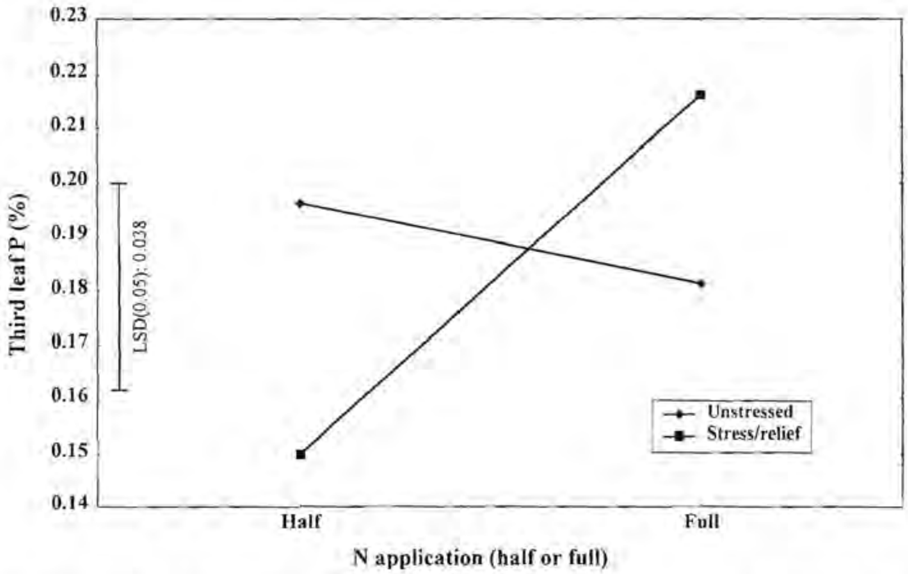


Figure 5.7. Third leaf P (%) as affected by the interaction between N applied and moisture stress treatment.

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

The main effects associated with the analysis of variance (four moisture stress treatments, four sampling dates and 15 plant parts) indicated that moisture stress treatments (unstressed, stressed (early), stressed (late) and stress/relief) resulted in highly significant differences in mean plant K (%) values (Table 5.3), as did the partitioning of the plants into their various components. Sampling date had no significant effect on plant K (%). The mean plant K (%) values for the stressed (early), stressed (late) and stress/relief treatments were all significantly different from that of the unstressed cane. The plant K (%) associated with the stress/relief treatment was not significantly different from that of the stressed treatments which indicated little evidence of recovery of the K in the cane following stress relief. In relation to the various plants, it was found that although the plant K (%) values generally declined with increasing leaf and sheath number, and the K (%) content of the sheaths was significantly higher than that of the corresponding lamina. The highest K (%) value was associated with the sheath of the second leaf. The partitioned third leaf indicated that the K content of lamina decreased from the base to the tip ie. L3R > L3La > L3T, and that the midrib had the lowest K concentration of the lamina parts.

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

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

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

In further consideration of the whole plant, the analysis of variance indicated a significant interaction between moisture stress x sampling date (Figure 5.8). Although the plant K (%) values associated with the stressed (early) were generally lower than those of the unstressed treatment, the differences were either not significant or only marginally significant. The same trend was generally apparent when the K (%) values of stressed (late) treatment were compared to those of the unstressed cane. Initially (first sampling: 100 days after planting and ten days after water was withheld), the K (%) value associated with the stress/relief treatment was significantly lower than that of the unstressed cane, but not significantly different from the values associated with the other stressed treatments. However, with stress relief the K (%) values increased with time and by the fourth sampling (day 130 after planting and 20 days after re-watering), there was no significant difference between the K (%) associated with the unstressed and stress/relief treatments.

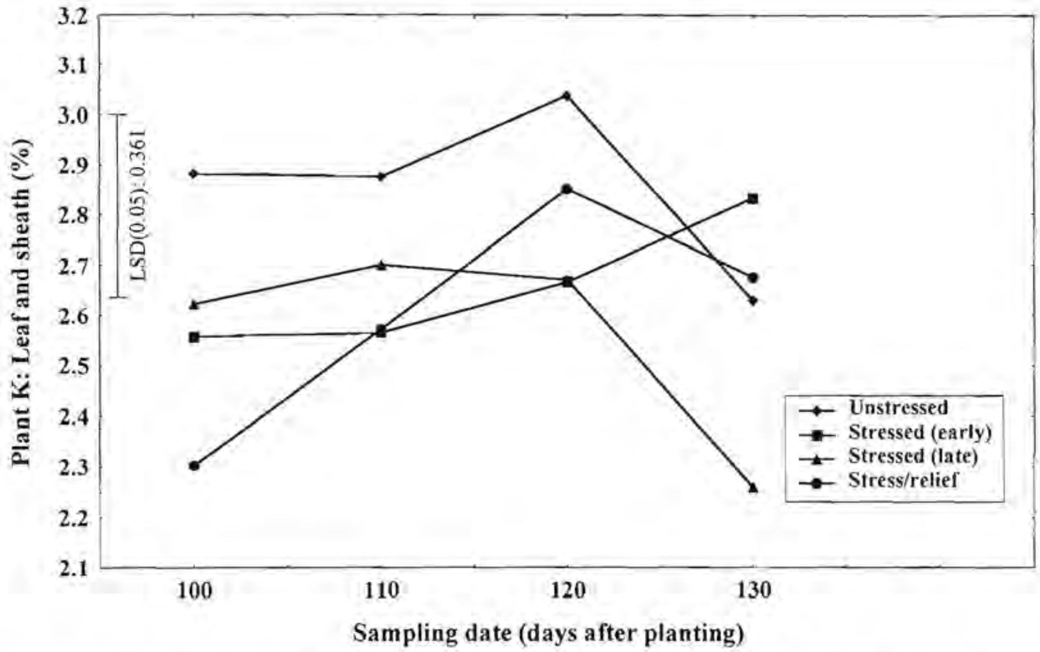


Figure 5.8. The interactive effect of moisture stress treatment and sampling date on total plant K (%).

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

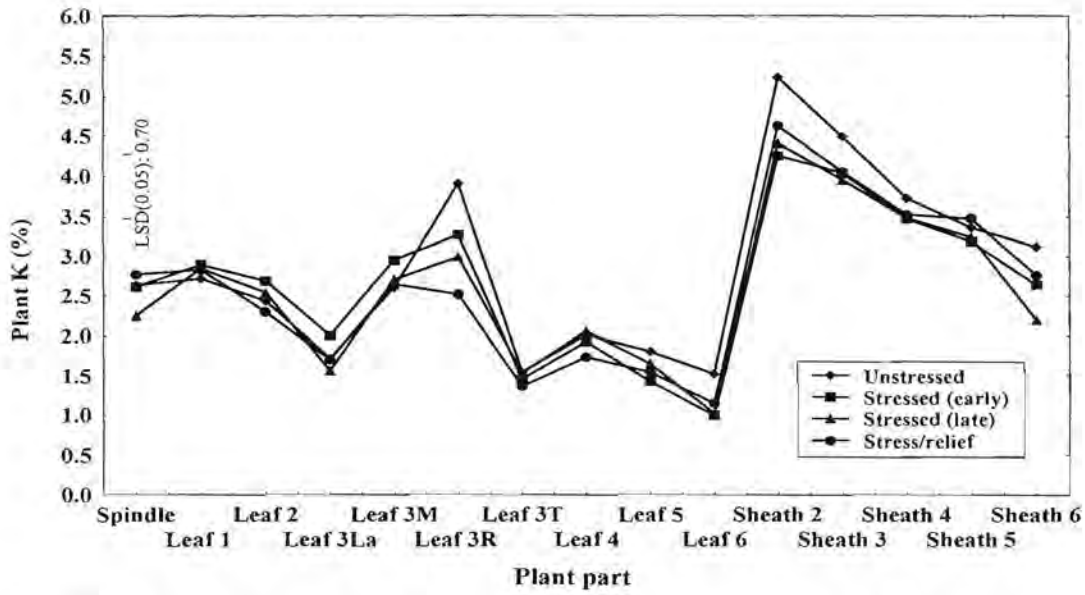


Figure 5.9. Plant K (%) of the different plant parts associated with the four moisture stress treatments.

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

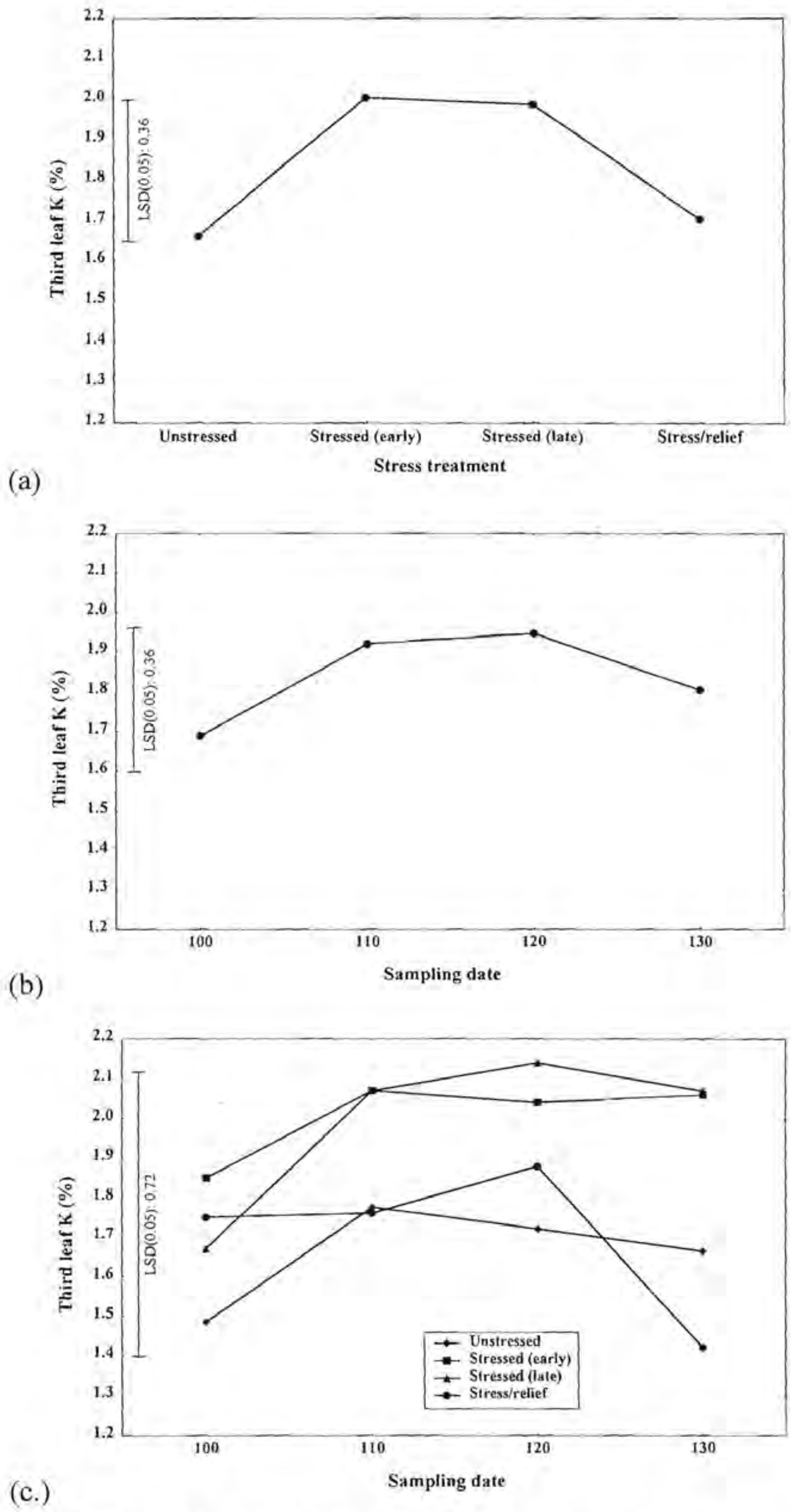


Figure 5.10. Third leaf K (%) as influenced by moisture stress treatment (a), sampling date (b) and the interaction between moisture stress and sampling date (c).

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

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

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

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

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

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

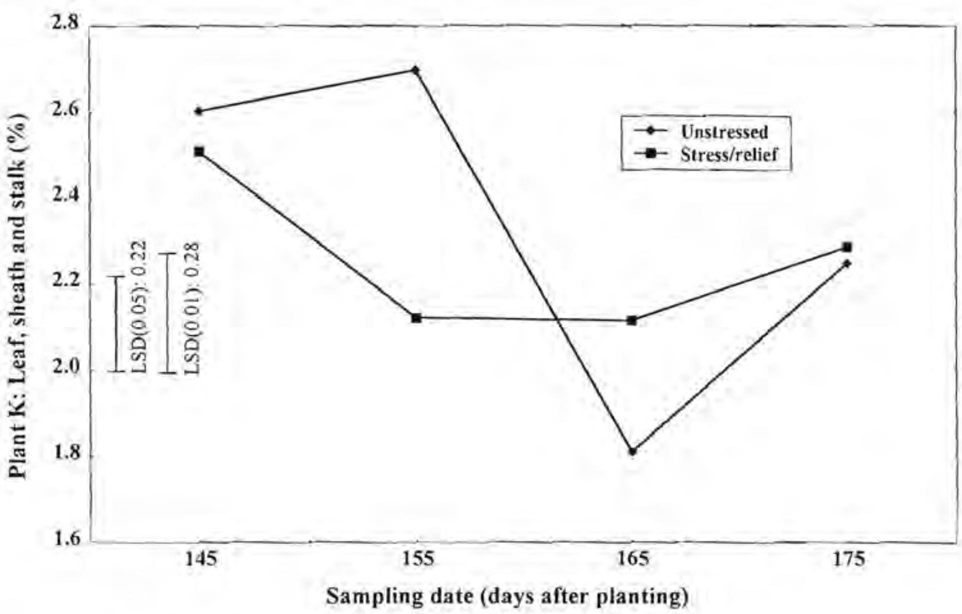


Figure 5.11. Total plant K (%) as influenced by moisture stress and sampling date.

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

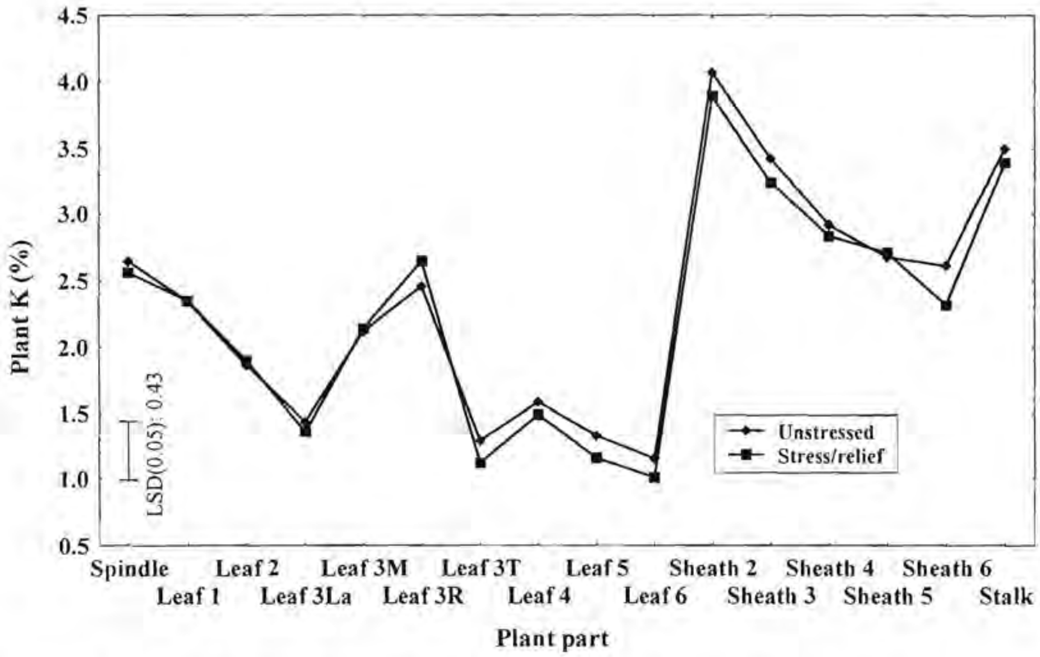


Figure 5.12. Plant K (%) of the different plant parts associated with the two moisture stress treatments.

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

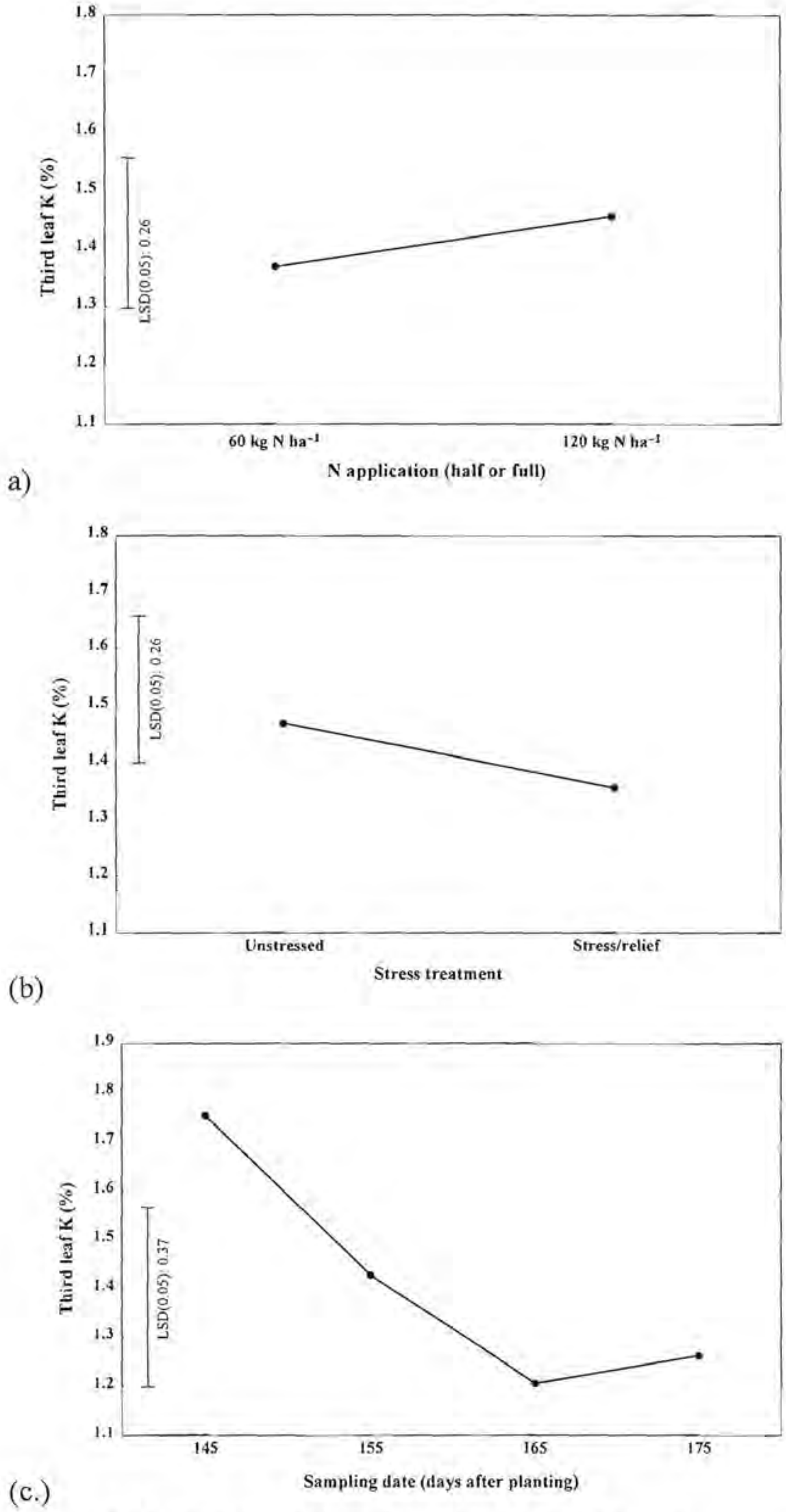


Figure 5.13. Third leaf K (%) as influenced by N application rate (a), moisture stress treatment (b) and sampling date (c).

5.4. Conclusions

The following conclusions were drawn:

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

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

Chapter 6.

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

6.1. Introduction

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

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

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

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

6.2. Procedure

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

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

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

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

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

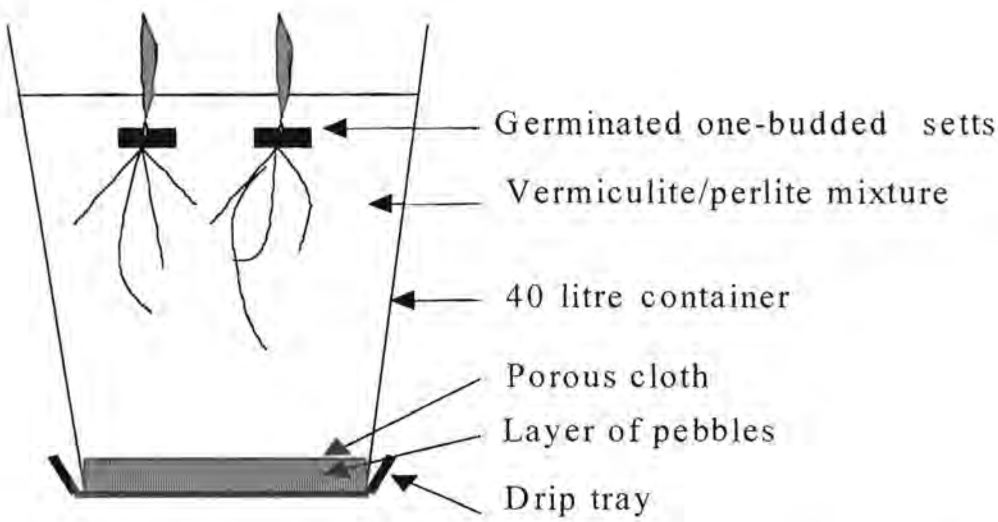


Figure 6.1 A diagrammatic representation of the planted containers used in the investigation.

Table 6.1. Nutrient quantities applied to each pot

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

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

6.2.1. Experimental design

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

The moisture stress treatments were as follows:

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

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

The harvest (sampling) dates were as follows:

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

6.2.2. Experimental details

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

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

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

where:

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

6.3. Results and discussion

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

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

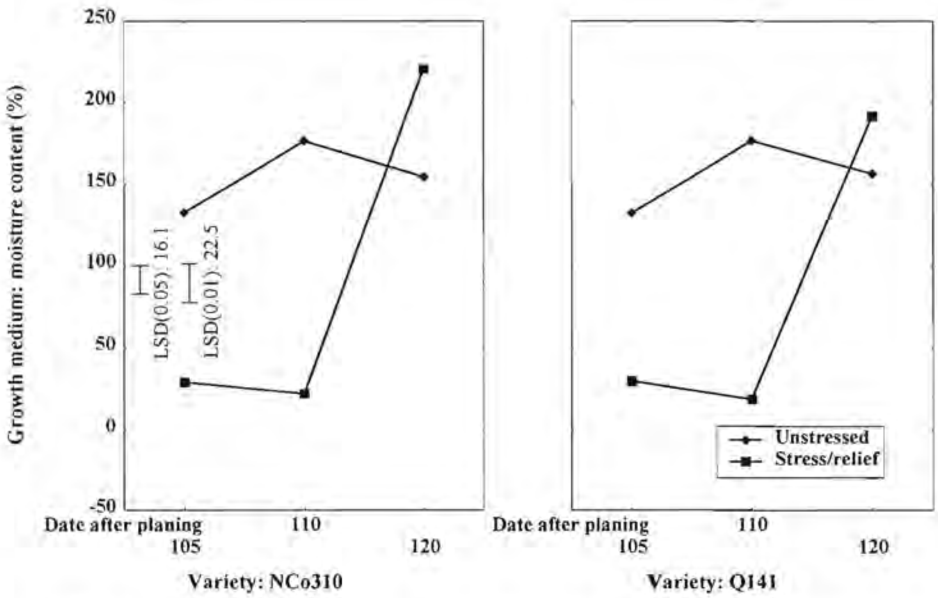


Figure 6.2. Mean moisture content values (%) of the vermiculite/perlite growth medium associated with the unstressed and stress/relief treatments for the two varieties (NCo310 and Q141) during the 20-day sampling period.

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

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

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

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

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

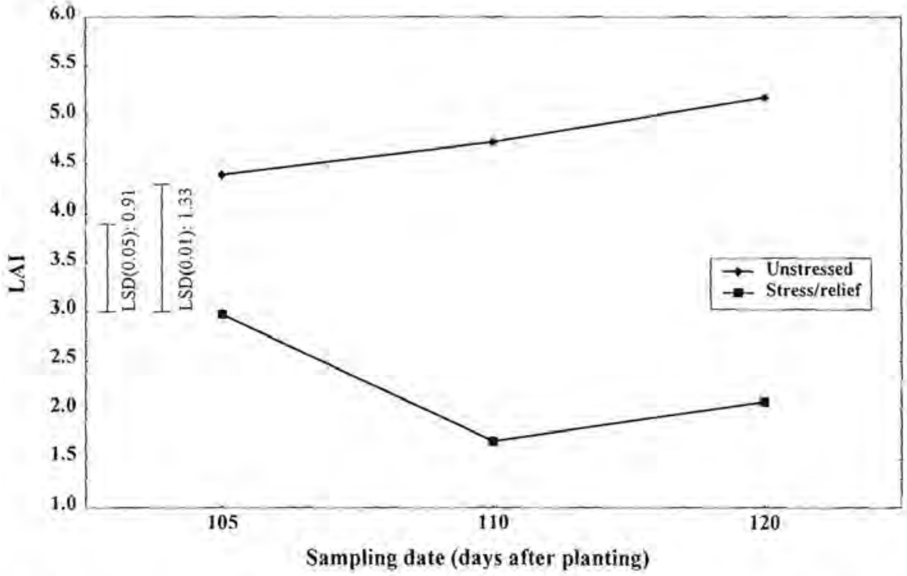


Figure 6.3. Interactive effect of moisture stress treatment (unstressed and stress/relief) and sampling date (days after planting) on LAI.

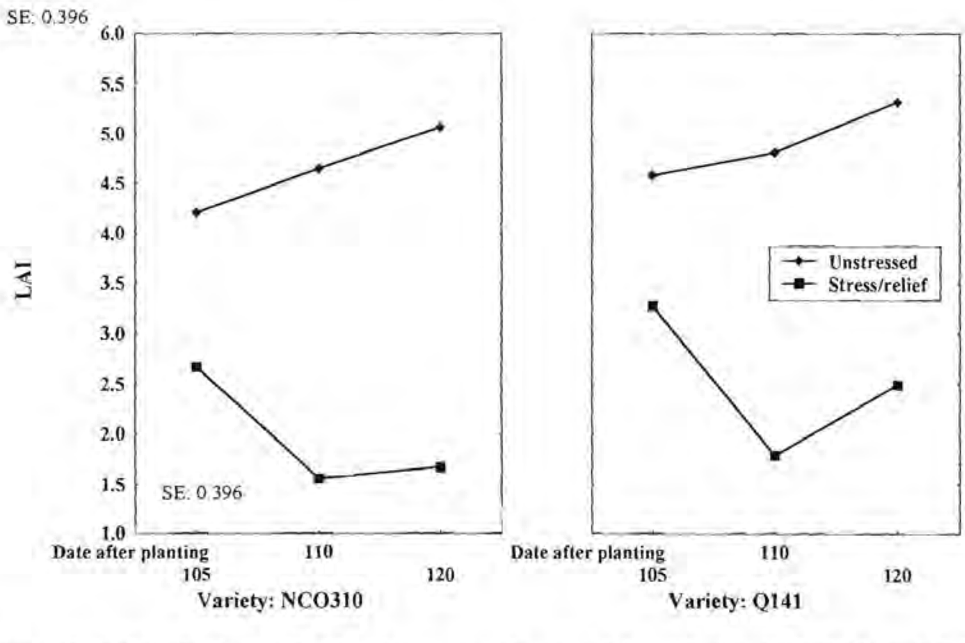


Figure 6.4. Similarity in LAI values associated with varieties NCo310 and Q141 under unstressed and stress/relief conditions.

When the main effects associated with biomass accumulation were considered it was found that, as with LAI, no varietal differences were apparent. However, both moisture stress treatment (unstressed and stress/relief) and sampling date (days after planting) resulted in significant differences in dry matter production (Table 6.2). In particular, the dry matter yield associated with the stress/relief treatment was significantly lower ($P<0.01$) than that of the unstressed cane.

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

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

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

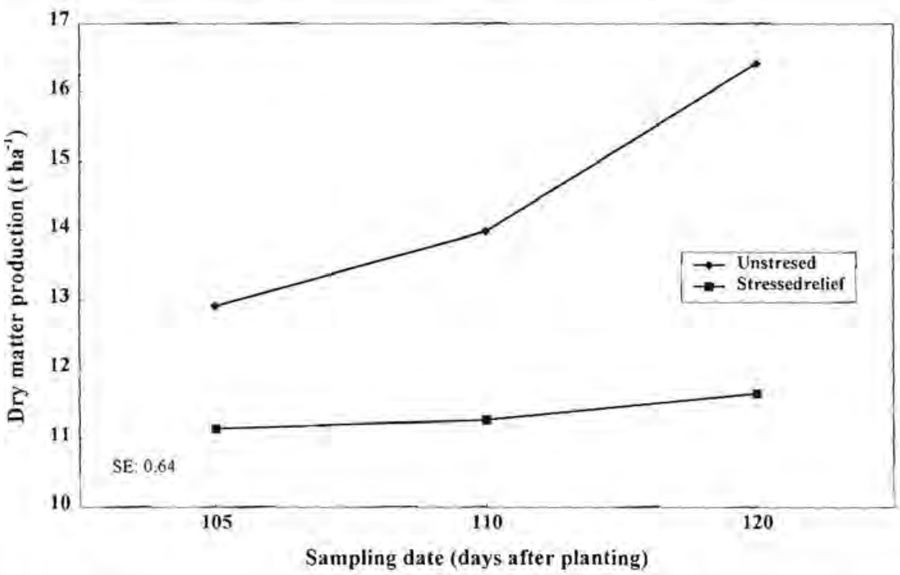


Figure 6.5. Dry matter production as affected by moisture stress and sampling date.

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

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

Third leaf N (%)

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

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

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

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

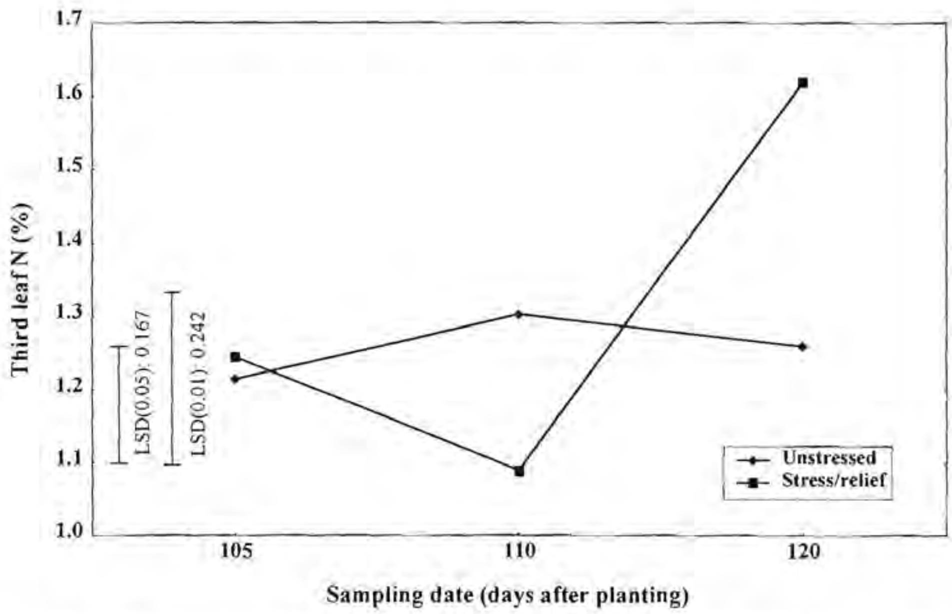


Figure 6.6. The interactive effect of moisture stress and sampling date on third leaf N (%) values.

Third leaf P (%)

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

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

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

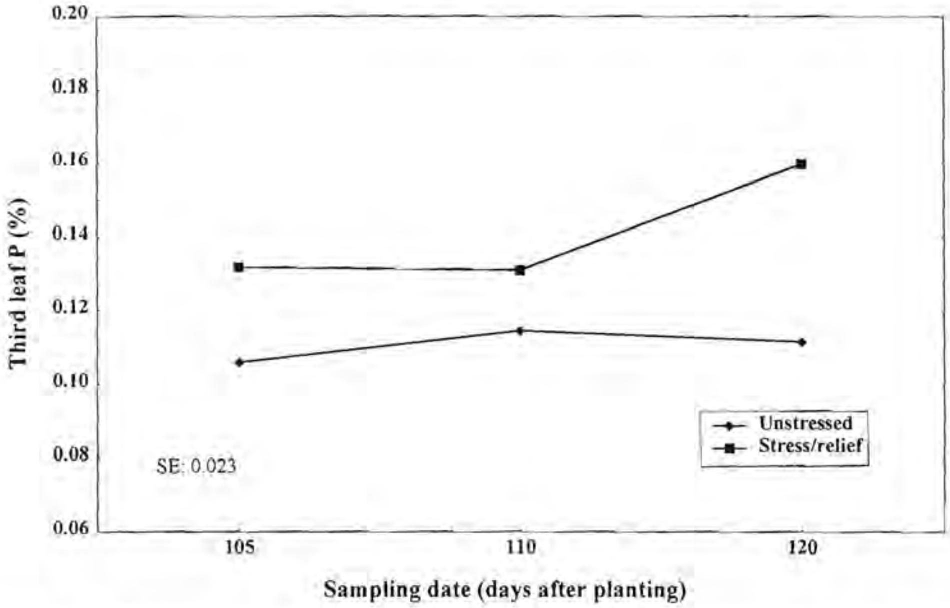


Figure 6.7. Third leaf P (%) values associated with the unstressed and stress/relief treatments on the various sampling dates.

Third leaf K (%)

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

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

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

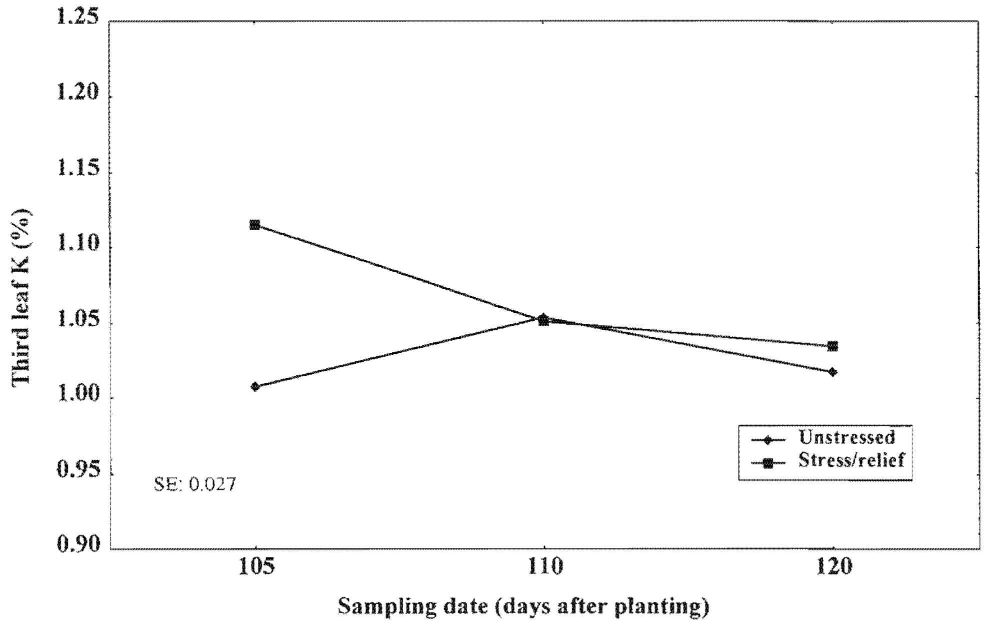


Figure 6.8. Third leaf K (%) values associated with the unstressed and stress/relief treatments on the various sampling dates.

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

As in the case of Trial 1 (Qld), withholding water (within the stress/relief treatment) resulted in the moisture content of the vermiculite/perlite growth medium being substantially depleted (Figure 6.9) and giving rise to conditions that would simulate "drought" effects. In the case of the unstressed treatment, the moisture content of the growth medium was kept above 150%. That the moisture content of the growth medium at 'field capacity' in Trial 2 (Qld) appeared to be slightly higher than that noted in Trial 1 (Qld) (Figure 6.2) was thought to be related to small differences in the ratio of vermiculite to perlite. However, due to the relatively large water holding capacity of the growth medium these differences were not considered important. In relation to the stress/relief treatment, rewatering on day 110 after planting increased the moisture content of the growth medium from about 18% to approximately 180%. The analysis of variance indicated that there were no significant differences in the moisture content of the growth medium associated with the two varieties.

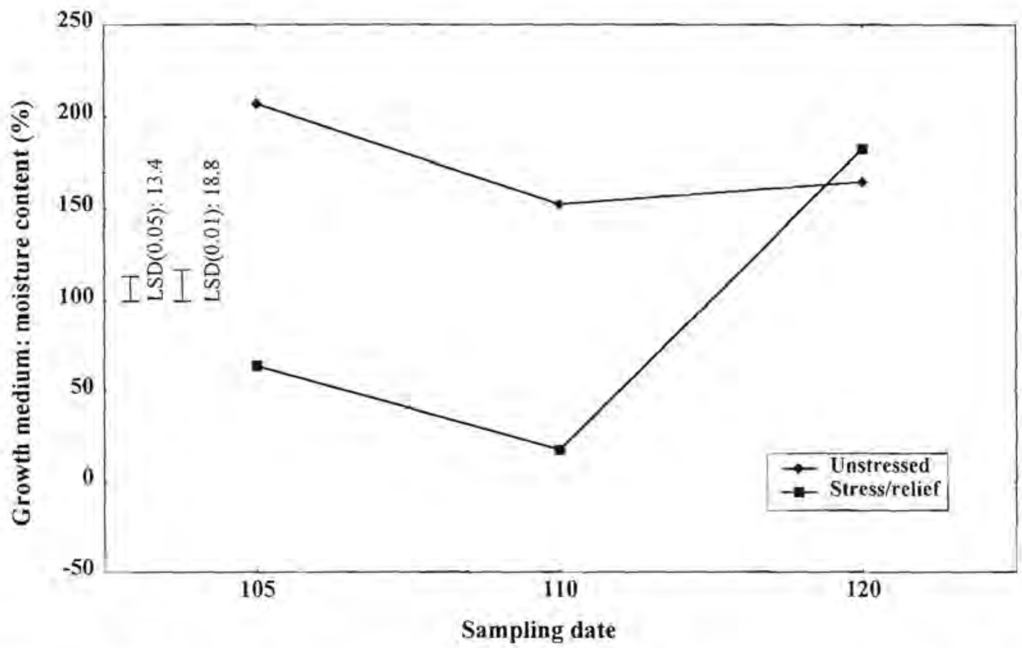


Figure 6.9. Mean moisture content values (%) of the vermicultite/perlite growth medium associated with the unstressed and stress/relief treatments during the 20-day sampling period.

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

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

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

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

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

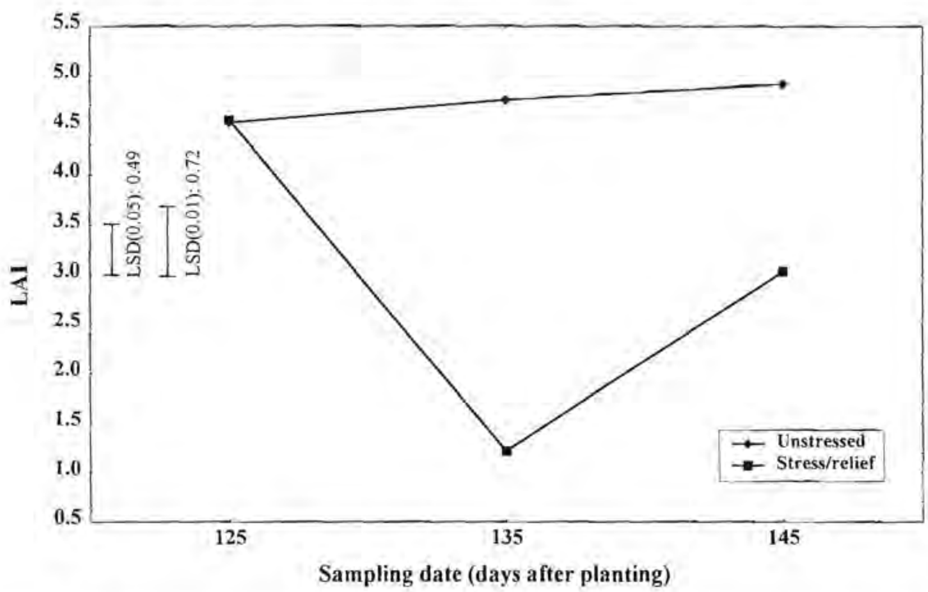


Figure 6.10. Interactive effect of moisture stress treatment (unstressed and stress/relief) and sampling date (days after planting) on LAI.

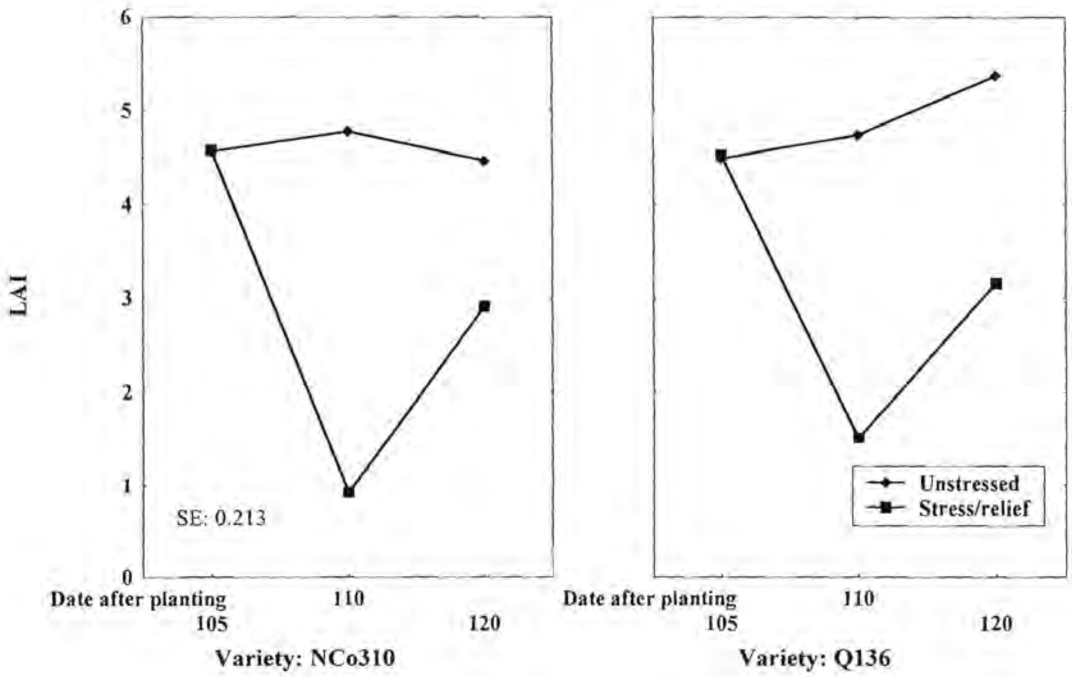


Figure 6.11. Plot of LAI versus sampling date under unstressed and stress/relief conditions for the two sugarcane varieties NCo310 and Q136.

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

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

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

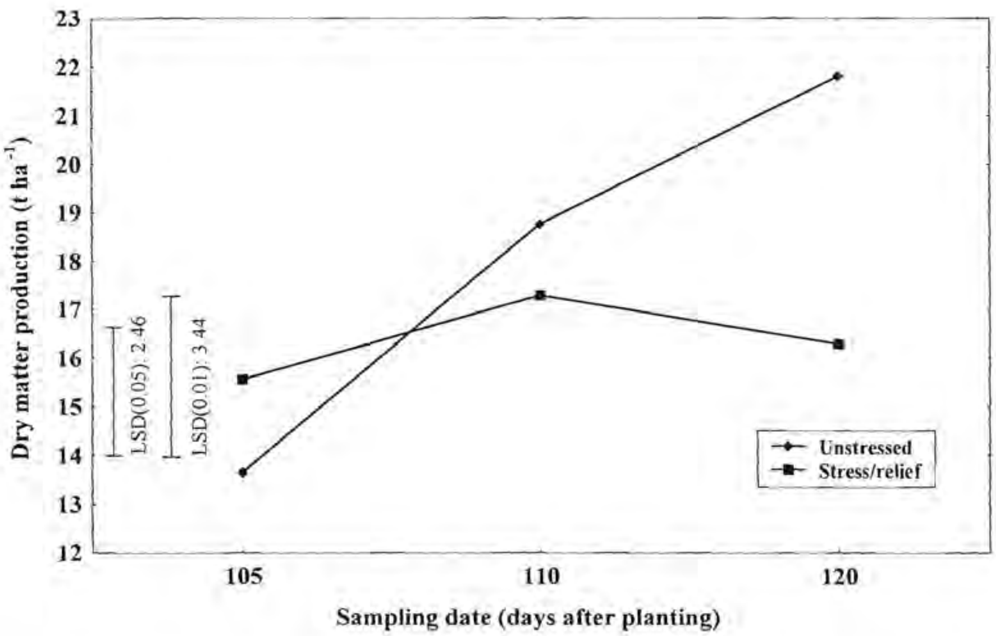


Figure 6.12. Dry matter production as affected by moisture stress and sampling date.

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

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

Third leaf N (%)

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

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

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

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

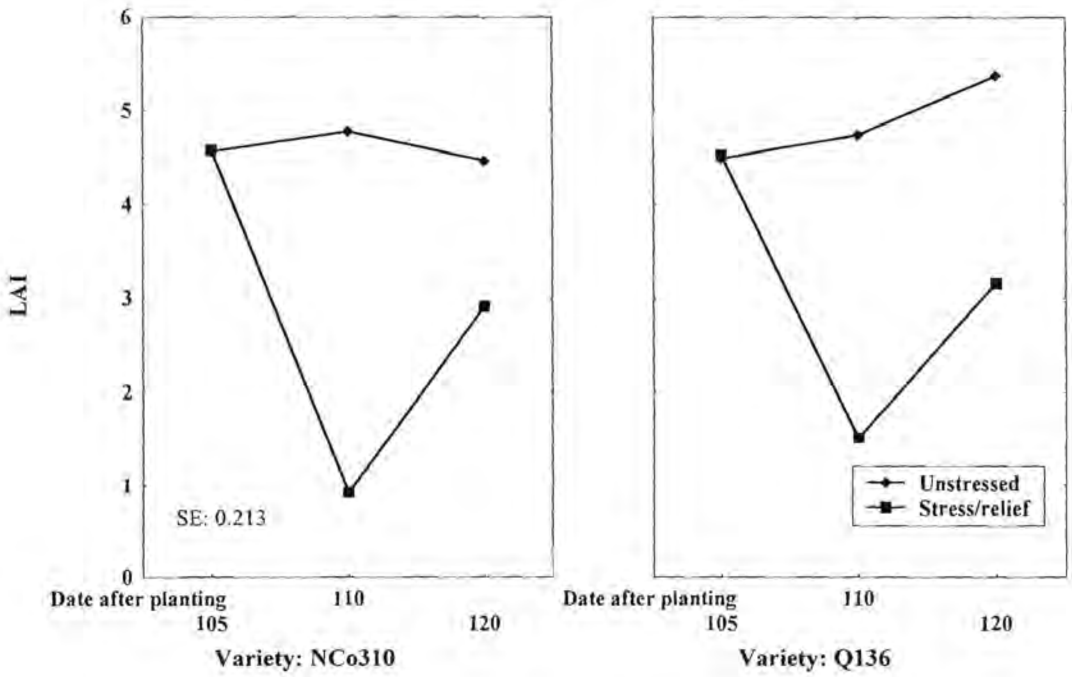


Figure 6.11. Plot of LAI versus sampling date under unstressed and stress/relief conditions for the two sugarcane varieties NCo310 and Q136.

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

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

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

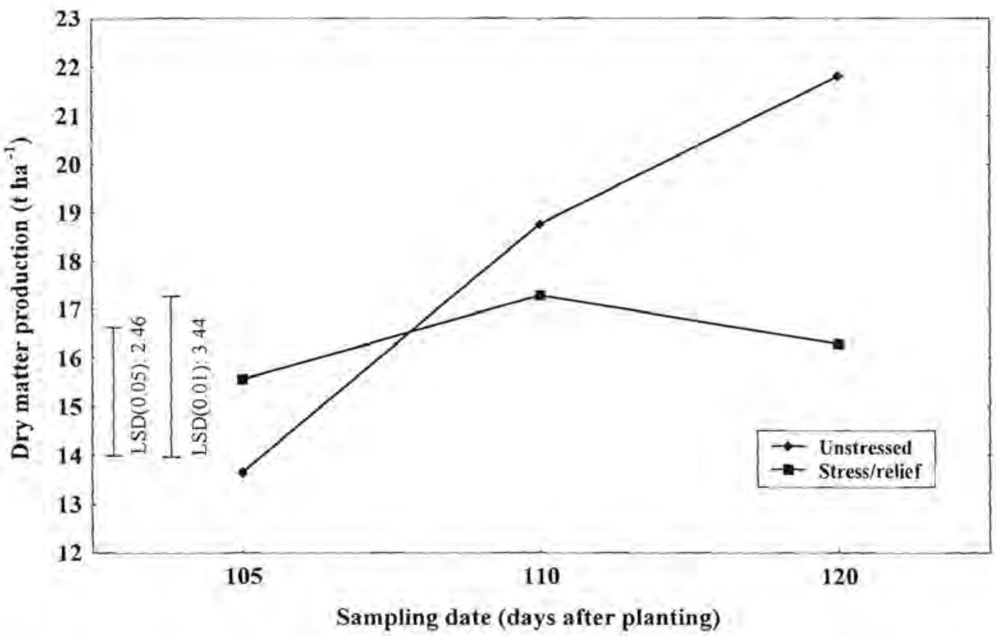


Figure 6.12. Dry matter production as affected by moisture stress and sampling date.

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

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

Third leaf N (%)

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

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

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

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

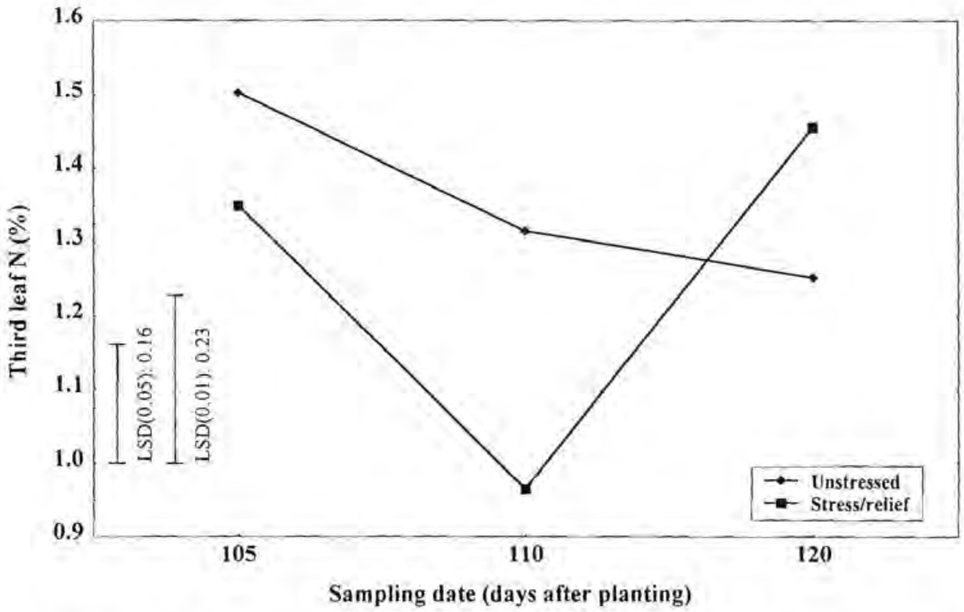


Figure 6.13. The interactive effect of moisture stress and sampling date on third leaf N values.

Third leaf P and K (%)

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

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

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

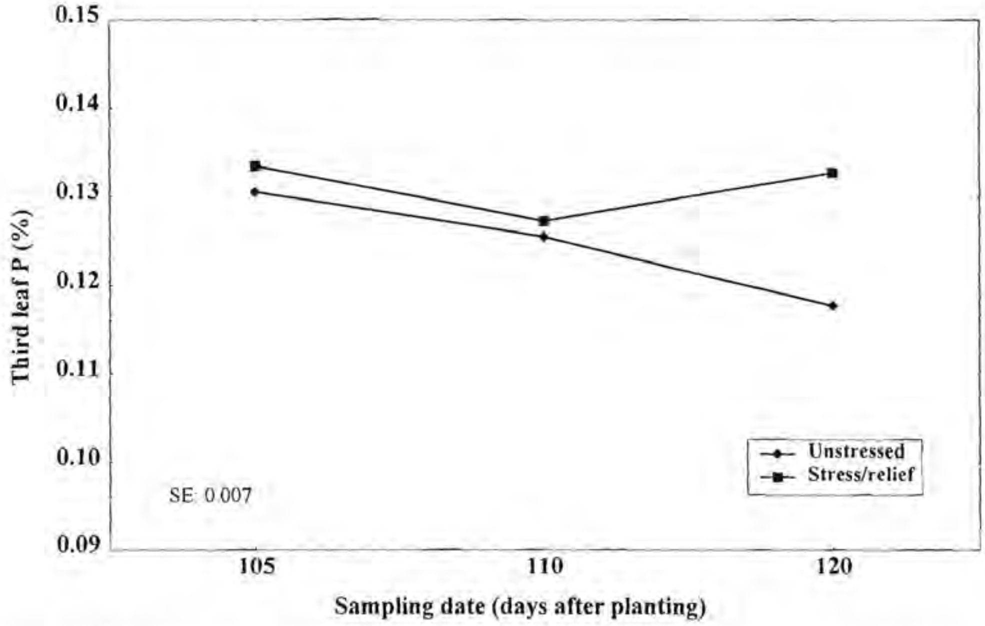


Figure 6.14. Third leaf P (%) values associated with the unstressed and stress/relief treatments on the various sampling dates.

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

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

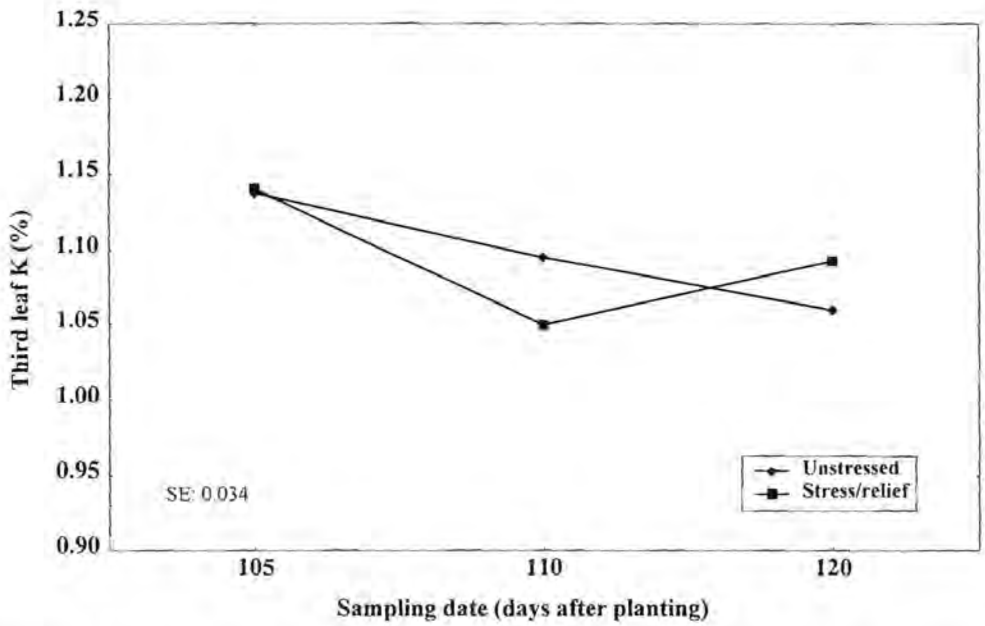


Figure 6.15. Third leaf K (%) values associated with the unstressed and stress/relief treatments on the various sampling dates.

6.4. Conclusions

The following conclusions were drawn:

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

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

Chapter 7.

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

7.1. Introduction

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

One option that was initially considered in this investigation was a "bio-chemical" moisture stress indicator that could be determined on the plant tissue at the time of analysis. As the accumulation of amino acids in various plant parts had been investigated by scientists in various sugar industries for drought tolerance, sugar quality, fertiliser management, etc (Rutherford, 1989; Chapman *et al*, 1996; Keating *et al*, 1999), the possibility soon fell towards proline which was widely reported to accumulate under moisture stress condition in a number of agricultural crops (Rao and Asokan, 1978; Rutherford, 1989; Irrigoyen *et al*, 1992; Steyn and Rossouw, 1995). However, from literature it was established that although proline could possibly be useful in mechanisms involved with drought tolerance in sugarcane, it would not serve as a robust moisture stress indicator for use with nutrient leaf analysis data. The reasons for this included reports that proline accumulation could occur due to other plant stresses apart from moisture stress (Aspinall and Paleg, 1981), be affected by crop age, sampling season and by leaf K concentration (Rutherford, 1989), and vary according to variety (Rao and Asokan, 1978). In addition, it appeared that proline concentration in the leaf tissue was affected by conditions after sampling as shown by Rutherford (1989) in simulating 'drought conditions' using polyethylene glycol solutions. Although accumulation of proline in excised leaf could be prevented by immediately freeze-drying the leaf tissue (Anon., 1994 (b)) this facility would be unavailable to growers during routine leaf sampling.

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

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

In light of the above, it was deemed necessary to find a moisture stress indicator that could allow easier interpretation of leaf analysis data, but would also be appropriate for general and practical use in the industry. Of particular interest was the possible use of the dry masses of the various plant components (spindle, leaf, sheath and trash) expressed as a percentage of their wet masses (D%W). This would not only be readily available, but could easily be incorporated into leaf sampling routines.

7.2. Procedure

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

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

7.3. Results and discussion

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

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

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

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

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

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

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

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

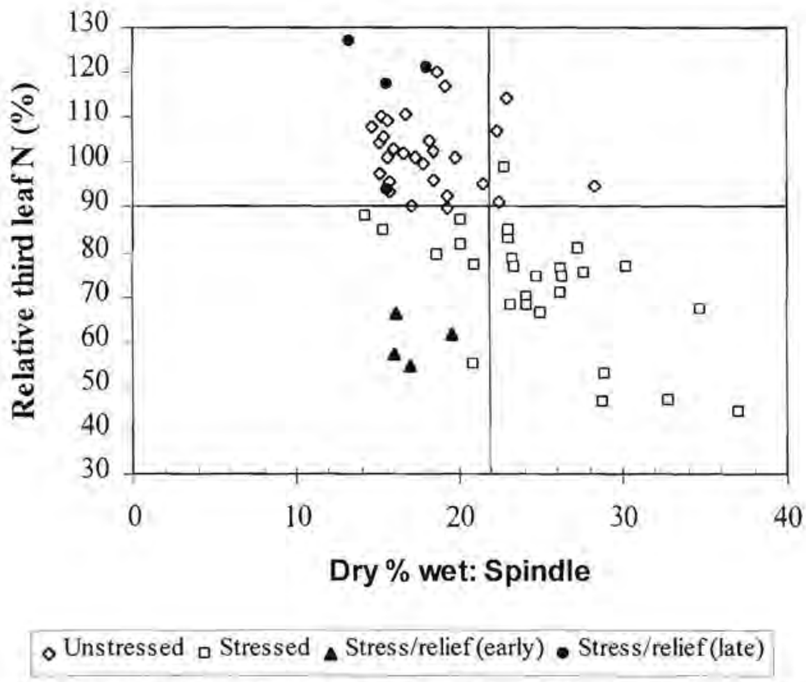


Figure 7.1. Relative third leaf N (%) plotted against D%W (spindle). D%W value of below 22% would indicate unstressed conditions in the spindle.

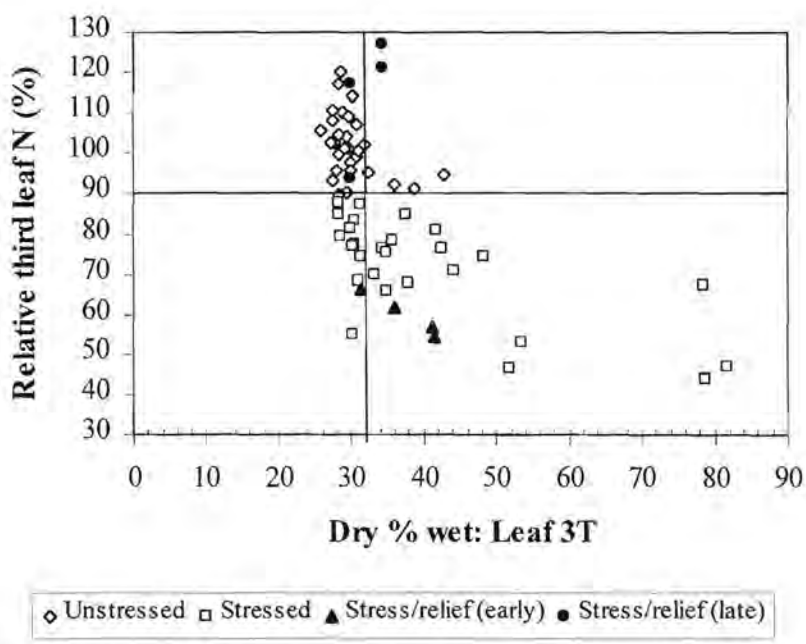
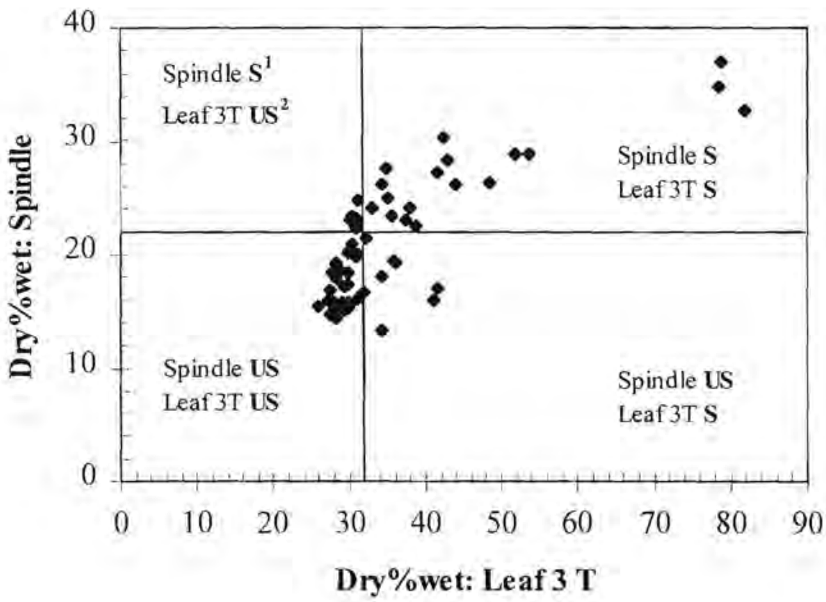


Figure 7.2. Relative third leaf N (%) plotted against D%W (L3T). D%W value of below 32% would indicate unstressed conditions in the third leaf.

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



[1]S = Stressed [2]US = Unstressed

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

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

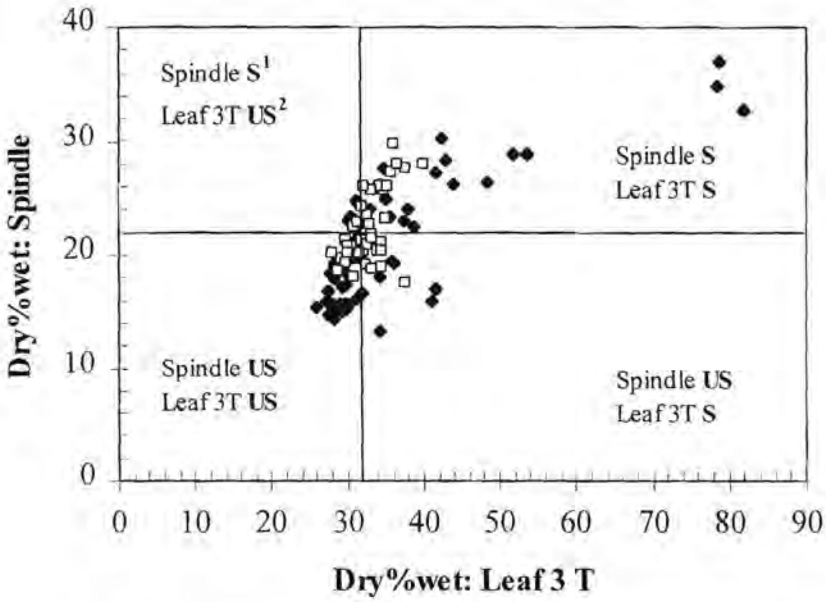


Figure 7.4. Validated plot of D%W (spindle) against D%W (L3T) indicating that both the D%W (spindle) and the D%W (L3T) of 22 and 32% respectively are suitable for general use with sugarcane.

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

Mean relative third leaf N (%) values (Trial 1) plotted as a function of D%W: L3T (Figure 7.5) showed that the two quantities were reasonable well correlated (r^2 = 0.656), and that the resulting regression equation (Equation 2) provided a means of determining the relative third leaf N value for a given D%W(L3T) value. In addition the calculated relative third leaf N value would enable the estimation of an unstressed third leaf N value from a third leaf N value affected by moisture stress, as shown in the example below.

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

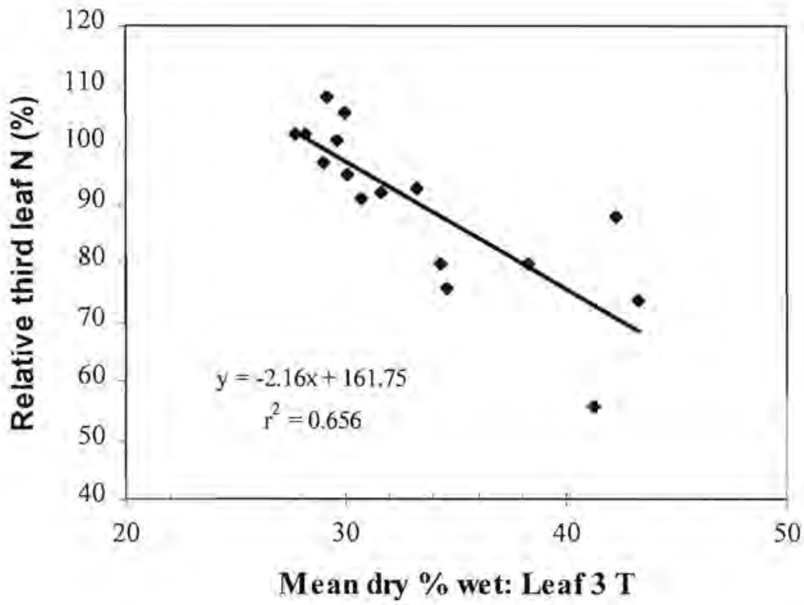


Figure 7.5. Relationship between relative third leaf N (%) and mean D%W (L3T).

Example:

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

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

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

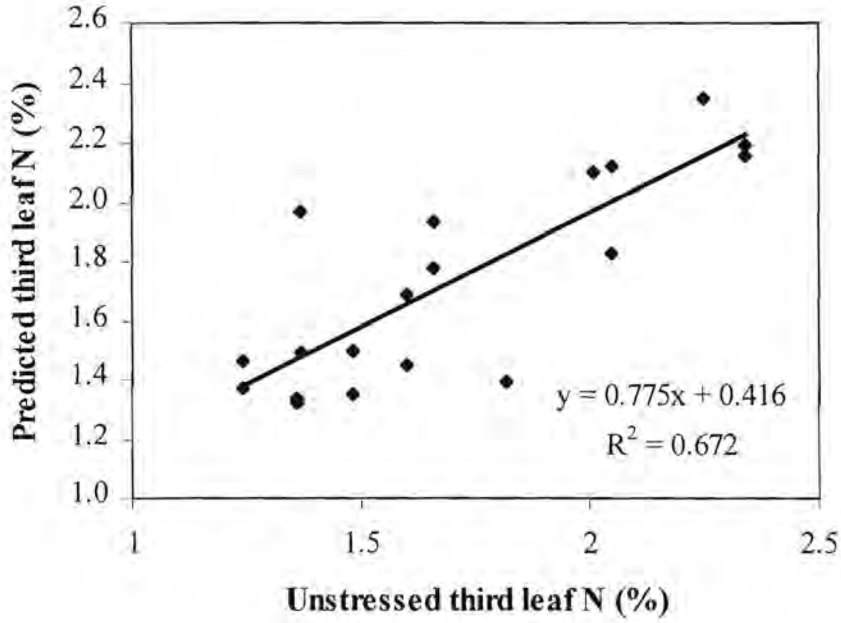


Figure 7.6. Predicted unstressed third leaf N (%) values plotted against actual unstressed third leaf values from Trials 2 and 2 (Qld).

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

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

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

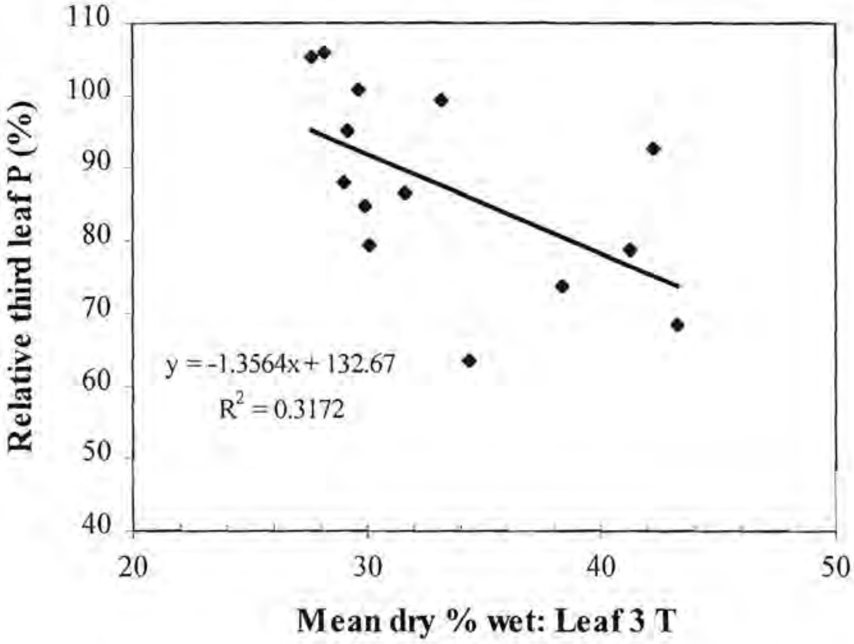


Figure 7.7. Relationship between relative third leaf P (%) and mean D%W (L3T).

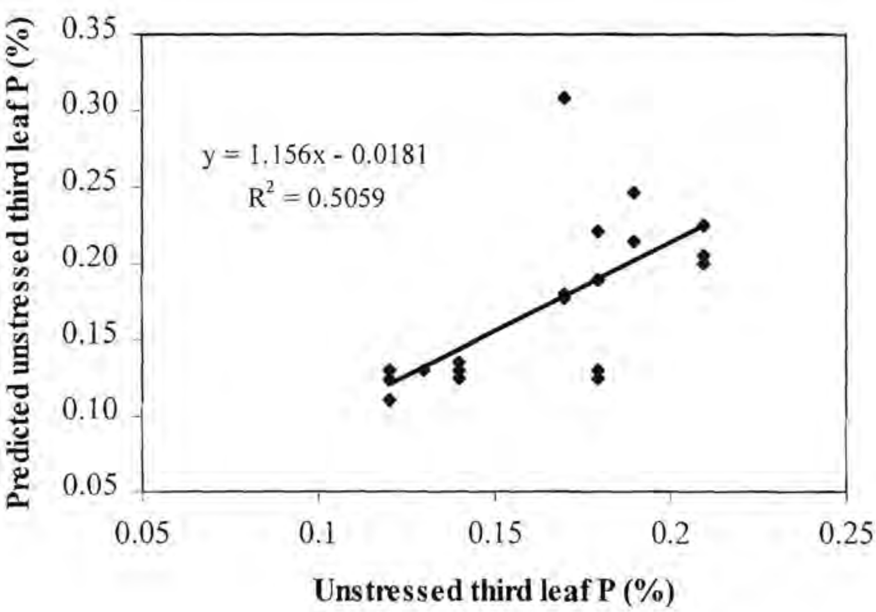


Figure 7.8. Predicted unstressed third leaf P (%) values plotted against actual unstressed third leaf P values from Trials 2 and 2(Qld).

7.4. Conclusions

The following conclusions were drawn:

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