A REMEDY FOR DEALING WITH MOISTURE STRESS CONDITIONS DURING SUGARCANE LEAF SAMPLING.

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Abstract

Currently it is recommended that sugarcane only be leaf sampled when it is actively growing, as third leaf N values are, in particular, affected by moisture stress conditions. As drought conditions relatively often affect the South African sugar industry, they impose constraints that severely curtail regular leaf sampling. This investigation was aimed at finding a moisture stress indicator that could be used to identify moisture stress at the time of sampling, and provide a means of assessing or interpreting leaf analysis data (particularly N) under such conditions.

It was found that the dry mass of the top sections of the third leaf laminae (between the middle 300mm section (used for chemical analysis) and the leaf tip) expressed as a percentage of their wet mass (D%W(L3T)) used in combination with the dry mass of a sample of spindles (from the same plants) expressed as a percentage of their wet mass (D%W (Sp)), provided such an indicator. D%W (L3T) values of less that 32% in combination with D%W (Sp) values less than 22% would indicate unstressed conditions in sugarcane at the time of sampling. D%W (L3T) values greater than 32% in combination with D%W (Sp) values above 22% would indicate stressed conditions. D%W (L3T) values above 32% in combination with D%W (Sp) less than 22% would indicate stress-relieved conditions but with inadequate recovery of the third leaves (moisture and nutrients). In cases where D%W (L3T) indicated moisture stress conditions, estimation of 'unstressed' third leaf N values corresponding to third leaf N values affected by moisture stress (as quantified by a D%W (L3T) value) was found to be possible, using a regression equation (r^2=0.656) that linked relative third leaf N values (actual third leaf N values expressed as a percentage of baseline values) to D%W(L3T). Although the estimation of 'unstressed' third leaf P values corresponding to third leaf P values affected by moisture stress was also found to be possible, the appropriate regression equation was weaker that that associated with the third leaf N values.

The proposed use of D%W (L3T) and D%W (Sp), together with the regression equations relating D%W (L3T) to relative third leaf nutrient values provides a useful remedy for dealing with moisture stress conditions during leaf sampling. The substantially eased constraints on leaf sampling will hopefully encourage renewed, and possibly greater, use of leaf analysis for better nutrient management in sugarcane production.

Introduction

It is currently recommended that sugarcane only be leaf sampled when it is actively growing, as third leaf N values, in particular, are adversely affected by moisture stress conditions (Halais, 1962; Evans, 1965; Gosnell and Long, 1971). This 'avoidance technique' has meant that leaf samples are only collected when either enough well distributed rainfall has occurred or sufficient irrigation has been applied to preclude any moisture stress effects at, or prior, to sampling (Schroeder *et al*, 1992). Such prerequisites have imposed constraints that have severely curtailed or sometimes precluded leaf sampling in the South African sugar industry which is relatively often affected by drought conditions (Singels and Bezuidenhout, 1999). Even in good years, uneven rainfall distribution has lead to moisture stress conditions that have affected third leaf nutrient values (Schroeder *et al*, 1993). However, recent studies have led to a better understanding of the interaction between moisture stress, plant growth and the nitrogen content of young sugarcane (Schroeder *et al*, 2000). As a result, it was considered appropriate to investigate the development of a robust 'moisture stress indicator' that could be used when interpreting leaf analysis data irrespective of moisture status.

Although various options were considered, such as the use of a "bio-chemical" moisture stress indicator and/or commonly used agronomic measurements, they failed to meet the criteria of being robust and appropriate for general and practical use in South Africa or any other world sugar industry. For instance, proline (an amino acid) which was reported to accumulate under moisture stress conditions in a number of agricultural crops (Rao and Asokan, 1978; Rutherford, 1989; Irrigoyen *et al*, 1992; Steyn and Rossouw, 1995), was found to be unsuitable as a moisture stress index as there is evidence in literature that this amino acid could accumulate as a result of 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) this facility would be unavailable to growers during routine leaf sampling. Commonly used agronomic measurements such as growth rate and leaf area index (LAI), although suitable for assessing moisture stress conditions in glass-house experiments and field trials, would not be considered appropriate for on-farm usage, nor for rapid assessment of moisture stress conditions.

In light of the above, it was deemed necessary to find a moisture stress indicator that was readily available and could easily be incorporated into leaf sampling routines. Of particular interest was the possible use of the dry masses of the various plant components (spindle, leaf, sheath and trash) expressed as percentages of their wet masses (D%W). The aim of this investigation was to assess these parameters as suitable indices for identifying moisture stress at the time of sampling and providing a means of interpreting leaf analysis data (particularly N) under such conditions.

Procedure

The data discussed here were obtained from four separate randomised pot trials (conducted under an automatic rain-shelter or in a glasshouse) to investigate the interaction of moisture stress, plant growth and the nutrient content (particularly N) of young sugarcane (Table 1).

Various sugarcane varieties (NCo376 (Trials 1 and 2) and NCo310, Q136 and Q141(Trials 3 and 4) were grown in large 80 litre containers, with N fertiliser applications ranging from low to adequate (60 to 140 kg N ha^{-1}). Moisture stress treatments included unstressed, moisture stress (applied by withholding water once the cane reached at least three months of age) and stress/relief conditions (applied by withholding water once the cane reached at least three months of age, followed by re-watering after a set period). The pots were regularly weighed and plant growth measured to record the effect of moisture stress. The young plants were serially harvested at 10 day intervals following imposition of the moisture stress treatments. Total above ground yields were determined (not presented here) for each container prior to partitioning of the plants into samples corresponding to the spindle and different leaf numbers. The separate components were dried and weighed prior to chemical analysis in the laboratory. The dry masses of the spindle and leaves 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 middle 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 (Schroeder *et al*, 2000), 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 Trials 1 and 3 were separated from the data from Trials 2 and 4 to enable a validation step.
- A regressional analysis on the data from Trials 1 and 3 was used to determine the relationship between D%W (L3T) and relative third leaf N (%). Data from Trials 2 and 4 were used for validation purposes.

Table 1. Details of trials used in the investigation.

Trial	Site	Sugarcane variety	Soil/growth medium	N fertiliser application rate (kg N ha^{-1})	Moisture stress treatments	
					Status	Date applied (days after planting)
1	Rain-shelter: Central Field Station (Umhlanga Rocks)	NCo376	Red loamy sand	140	Unstressed Stressed (early) Stressed (late) Stress/relief	- 90 100 90 (re-watered from day 110)
2	Rain-shelter: Central Field Station (Umhlanga Rocks)	NCo376	Red loamy sand	120 (full rate)	Unstressed Stress/relief	- 140 (re-watered from day 165)
				60 (half rate)	Unstressed Stress/relief	- 140 (re-watered from day 165)
3	Glasshouse: Indooroopilly, Brisbane	NCo310	Vermiculite/perlite mix	120	Unstressed Stress/relief	- 100 (re-watered from day110)
		Q141	Vermiculite/perlite mix	120	Unstressed Stress/relief	- 100 (re-watered from day110)
4	Glasshouse: Indooroopilly, Brisbane	NCo310	Vermiculite/perlite mix	120	Unstressed Stress/relief	- 100 (re-watered from day110)
		Q136	Vermiculite/perlite mix	120	Unstressed Stress/relief	- 100 (re-watered from day110)

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 2). Two baselines were established for Trial 2 to reflect the full and half N application rates applicable in that case.

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

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 1).

The full set of relative third leaf N values (from all four trials) plotted against D%W of the spindle (Figure 1) and third leaf (Figure 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 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 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 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 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 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 2).

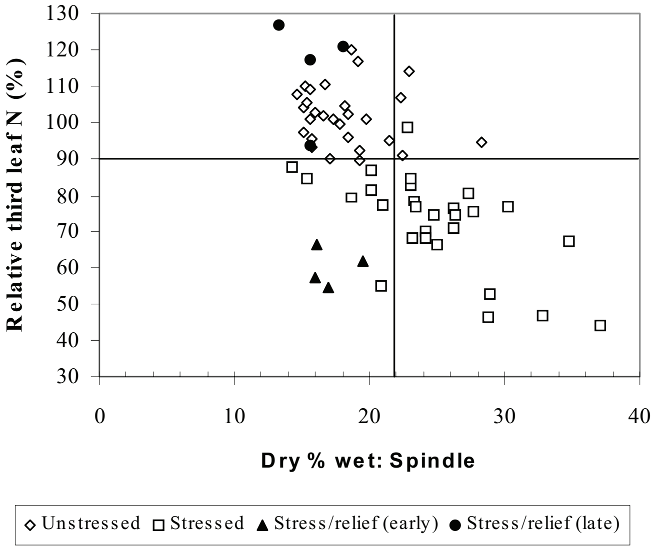

Figure 1. Relative third leaf N (%) plotted against D%W (spindle). D%W value of below 22% would indicate unstressed conditions in the spindle.

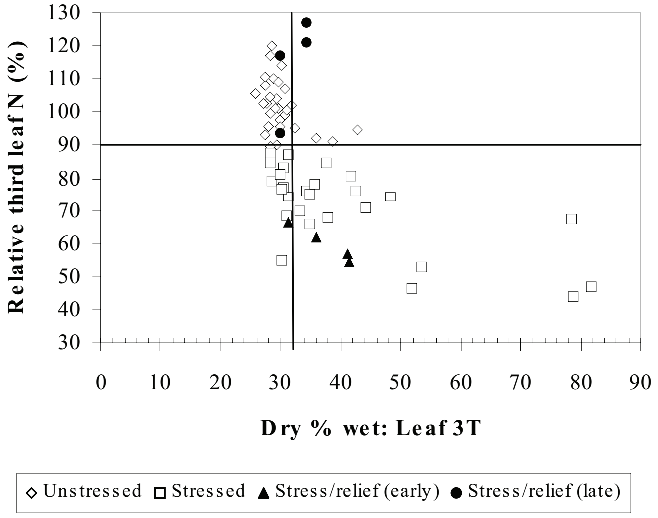

Figure 2. Relative third leaf N (%) plotted against D%W (L3T). D%W value of below 32% would indicate unstressed conditions in the third leaf.

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 are unaffected by moisture stress, both the D%W (spindle) and the D%W (L3T) values should be

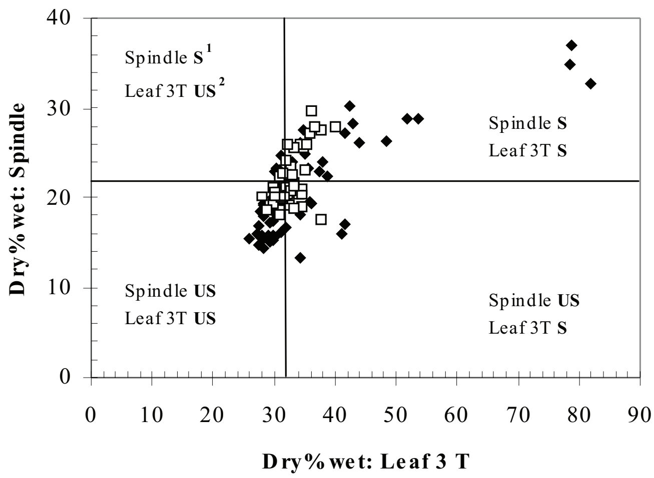

Figure 3. Plot of D%W (spindle) against D%W (L3T). Both D%W (spindle) and the D%W (L3T) values should be below their respective critical values ie. 22 and 32% respectively, to indicate unstressed conditions (open and closed symbols). Open symbols represent data from Trials 2 and 4 for validation purposes.

below their respective critical values ie. 22 and 32% respectively (Figure 3 – closed symbols).

For validation purposes, the D%W (spindle) and D%W (L3T) associated with Trials 2 and 4 were superimposed onto the graph in Figure 3 (open symbols). 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 circumstances and varieties other than NCo376 on which they were established. It was found that the combination of stressed spindles with unstressed third leaves did not occur (Figures 3). 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 4) showed that the two quantities were reasonably 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)

Example:

Third leaf N value = 1.44%

D%W (spindle) = 25%

D%W (L3T) = 36%

- Using Figure 3, 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 1) was tested using data from Trials 2 and 4, 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 5).

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). The relationship (Equation 3) based on Trial 1 data, was found to be relatively weak (Figure 6) with an r^2 value of 0.317. This probably reflects the fact that plant P is less sensitive than N to moisture stress (Samuels, 1965; Schroeder *et al*, 1993)

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

Table 2. Baseline third leaf N values related to relevant sampling dates, the associated mean third leaf N values and calculated relative third leaf N values.

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

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

However, a comparison of actual and predicted third leaf P values gave a correlation coefficient (r^2) of 0.506 (Figure 7).

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, 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. Midribs should not be removed from these top sections of the leaves. Similarly undried spindle samples (10 to 15 from across the field) 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 that that of the third leaf P values, both relationships offer a practical tool for allowing interpretation of nutrient values affected by moisture stress.

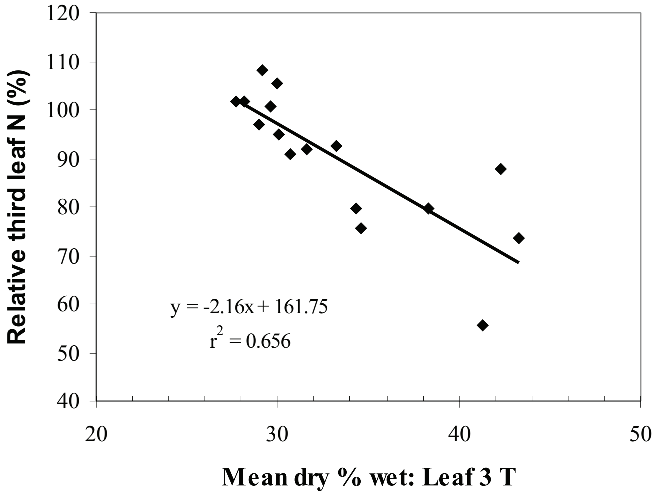

Figure 4. Relationship between relative third leaf N (%) and mean D%W (L3T).

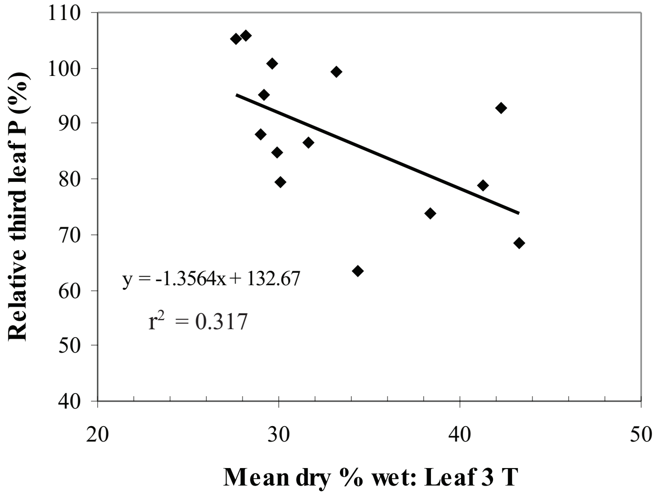

Figure 6. Relationship between relative third leaf P (%) and mean D%W (L3T).

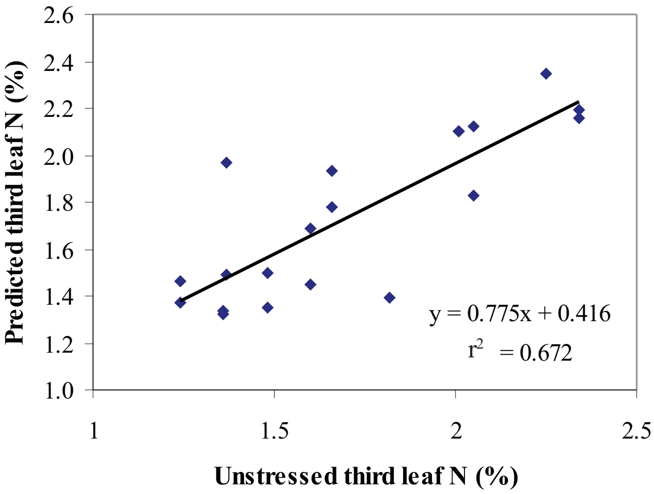

Figure 5. Predicted unstressed third leaf N (%) values plotted against actual unstressed third leaf values from Trials 2 and 4.

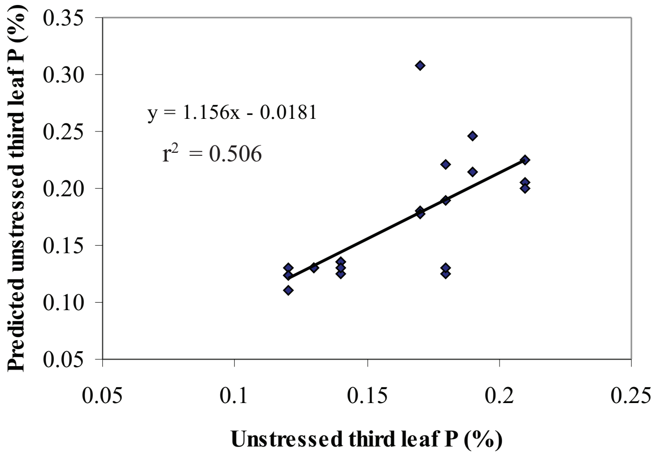

Figure 7. Predicted unstressed third leaf P (%) values plotted against actual unstressed third leaf P values from Trials 2 and 4.

- The proposed use of D%W (L3T) and D%W (Sp), together with the regression equations relating D%W(L3T) to relative third leaf nutrient values provides a useful remedy for dealing with moisture stress conditions during leaf sampling. The substantially eased constraints on leaf sampling will hopefully encourage renewed, and possibly greater use of leaf analysis for better nutrient management in sugarcane production.

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