

Final Report to SRDC

CSC 10s

Evaluating the leaf chlorophyll meter as a tool for nitrogen management in sugarcane.

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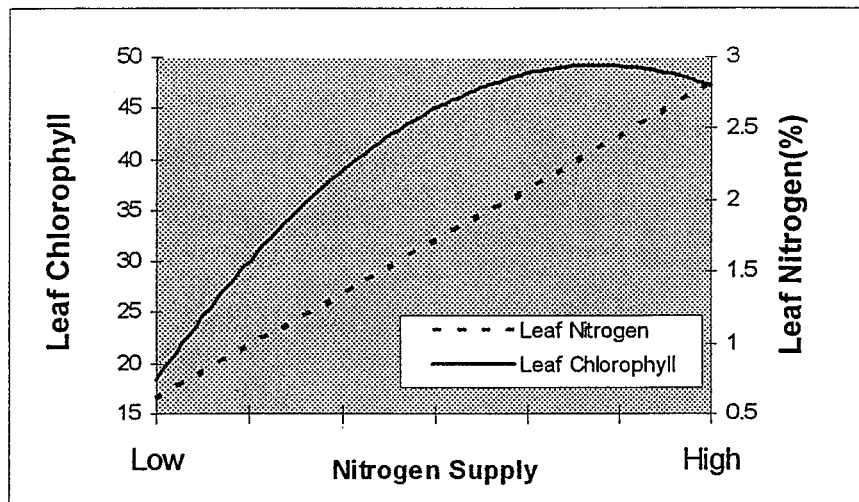
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Summary of Project

Nitrogen is an important constituent of chlorophyll, the compound that gives plants their green colour and the compound that absorbs radiation energy as the basis of plant growth. When nitrogen is in short supply, the chlorophyll content of leaves is reduced and in extreme cases, the leaves take on a yellow (or chlorotic) colour. There is an upper limit to leaf "greenness" and leaf nitrogen can continue to rise even when chlorophyll has reached a "plateau". These simple concepts are illustrated in the figure below.



This project was aimed at evaluating a portable leaf chlorophyll meter as a tool in nitrogen management of sugarcane. As chlorophyll is a critical component of the photosynthetic system, reductions in leaf chlorophyll may result in lower sugarcane growth rates and this may impact on final sugar yields. In addition, leaf nitrogen is expensive and time consuming to determine on an extensive basis. The notion was that the chlorophyll meter could provide a far simpler alternative to leaf analysis for the monitoring of nitrogen status of sugarcane crops.

The project consisted of measurements of leaf nitrogen and chlorophyll on sugarcane crops grown under a range of nitrogen regimes at locations in the Herbert, Bundaberg and northern NSW regions. Leaves at various levels in the canopy were measured and in the majority of instances samples of the bulk canopy were taken. Samples of the sap from the leaf mid-ribs were collected at the same time and analysed for nitrate using a rapid field test. In all 450 leaf N - chlorophyll meter comparisons were collected at various occasions over the November 1993 to June 1994 period.

The major findings were as follows ;

(1) The leaf chlorophyll meter is very rapid and convenient for field use. We estimate that the chlorophyll status of a block of cane could be assessed in 5 to 15 minutes, depending on how many locations in the block were to be sampled.

(2) Chlorophyll meter reading (a unitless output) was reasonably well correlated with the percentage of nitrogen in leaves on a dry weight basis. Relationships of the form denoted in the figure above meant that chlorophyll meter readings plateaued at values between 45 and 50.

(3) The third fully expanded leaf below the uppermost leaf with visible dewlap (referred to as leaf class 2 in this report) was found to be the most suitable leaf for testing on both practical and performance grounds.

(4) The data were somewhat equivocal on the impact of other site, cultivar and environmental factors of the relationships between chlorophyll meter reading and leaf N%. One site (BSES Bundaberg) did differ in the specific relationship, exhibiting lower leaf chlorophyll for a given leaf N% than the other sites. This difference largely disappeared when the leaf N data were expressed on a unit leaf area basis rather than a leaf weight basis. However data from another site (12 month old cane at Harwood) fell off the general relationship when expressed on such a basis.

(5) At this stage it appears the most reliable use of the leaf chlorophyll meter is on a relative basis. This is how it is used overseas (eg. the US corn industry - see Appendix 2). When the chlorophyll reading of a block of cane falls significantly below that of a "high N" control strip, there is a chance that yields will be reduced because of N deficiency. Under such circumstances, some benefit from a supplementary N fertiliser application is likely. In particular, there is a chance that N applied too late in the season may have no or detrimental effects on sugar yield. More definitive conclusions are limited by imperfect understanding of sugar yield determination in relation to the pattern of N supply.

(6) A comprehensive analysis of sugar yield response to N supply was outside the scope of this preliminary project. However, there was evidence from a comparison of chlorophyll data with final sugar yields, that a 5 to 10 % decline in relative chlorophyll reading in early growth (eg. November to February), (i.e. relative to values recorded in "high-N" controls) , could lead to sugar yield losses of a similar or greater magnitude.

(7) No useful role for the sap nitrate test on sugarcane crops was identified in this study.

(8) Care needs to be taken to avoid leaves affected by diseases, such as rust, when using the chlorophyll meter.

(9) The chlorophyll meter could play a useful role in N management of sugarcane in a situation where;

- Undiagnosed N deficiency during the early period of canopy development was limiting sugar yields and supplementary N fertilisation was a practical option to correct such deficiencies.
- or
- Where N rates were considered excessive and growers were prepared to experiment with reduced rates. Under such circumstances the chlorophyll meter

could be used to monitor the impact of the different N management regimes imposed.

We have no good way of knowing at present how likely either scenario is and what farmer reaction would be to a monitoring tool such as the chlorophyll meter. In relation to the former situation, there would be large economic benefits from the correction of any N deficiency. However, the current belief in the industry is that such deficiencies are rare under current management practise. In relation to the latter point, the extent of "over-fertilisation" with N is unknown at present. While there would be some small cost savings to growers if situations where N rates could be reduced without loss of production could be identified, the major benefits would accrue through reduced risks of excess nitrogen being lost to the environment.

We are not proposing a direct transfer of the chlorophyll meter technology to growers at this point in time, although we will explore the possibilities with individual growers as opportunities arise. Nitrogen research on sugar cane needs to work towards a "tool kit" that might include monitoring techniques, rules of thumb and simple "ready reckoners" aimed at improving the N management capability of growers and advisors. The chlorophyll meter will continue to be evaluated in association with other N research projects and it may end up as one item in this tool kit.

1. Background

Nitrogen fertiliser is a significant variable cost in sugar cane production and excess nitrogen can represent an environmental hazard if lost by leaching. Leaf nitrogen has been extensively used to monitor the adequacy of N supply for maximum sugar yield (Clements 1953, Baver 1963, Wood 1968, Gosnell and Long 1971, Meyer and Wood 1984). While leaf nitrogen affects cane and sucrose accumulation, the time delay and expense with laboratory determinations has been a disincentive to its use in practical management of N fertilisation of sugarcane.

Relatively inexpensive leaf chlorophyll meters (Minolta SPAD 502) have become available and these are currently being evaluated as a measure of leaf nitrogen to aid N management of corn in the USA (Follett et al. 1992, Schepers et al. 1992). The strategy employed in the Nebraska corn belt is to reduce the N fertilisation rate to something like 75% of the "standard", but supply full fertilisation rates to test strips located at intervals over the paddocks. Leaf chlorophyll is then monitored at regular intervals and if chlorophyll in the bulk field falls below that in the reference strips, supplementary N fertiliser applications are made (see Appendix 2 for further details). These measures are effective in reducing the incidence of "over-fertilisation" without loss of production and in doing so protect the groundwater that underlies the corn fields from nitrate contamination. This is possible because the probability of nitrate being leached increases sharply with each kg of N applied above crop requirements. In other words, because the last kg of N applied will effectively be the first kg to leach, small reductions in fertilisation rates can result in large reductions in leaching losses.

This project sought to import a chlorophyll meter into Australia and evaluate its technical performance in the assessment of leaf N status in sugar cane. While the prospects for integration of this technique into nitrogen management strategies for the industry are considered in this report, further work will be needed to identify the appropriate role in relation to other crop, soil and management monitoring approaches.

2. Objectives

This preliminary project aimed to evaluate the leaf chlorophyll meter in assessing the leaf nitrogen status of sugarcane crops in Queensland and NSW. Our objective was to quantify the relationship between leaf nitrogen and chlorophyll meter reading for;

- (1) Crops differing in N status.
- (2) Crops of different growth stage, cultivar and ratoon.
- (3) Crops grown in environments ranging from northern NSW to north Qld.

An additional objective was added after the project was initiated. Sap nitrate is used in the N management of cereal and horticultural crops (Elliot et al. 1987,1993, Olsen and Lyons 1984). The opportunity was taken to compare the sap nitrate test with the chlorophyll meter and leaf N content. An important difference between the tests is that leaf N% and Chlorophyll Meter readings assess the longer term history of N supply as reflected in leaf N or chlorophyll content. Sap nitrate reflects more of the current N environment on nitrate flows through the xylem and any free nitrate in the phloem or cytoplasm.

3. Technical Information

3.1 Chlorophyll Meter

The SPAD-502 chlorophyll meter determines the relative amount of chlorophyll present by measuring the transmittance of the leaf in two wavelength regions. Chlorophyll has transmittance peaks in the blue (600-700nm) and red (400-500nm) regions with no transmittance in the near-infrared region. The chlorophyll meter takes advantage of this characteristic of chlorophyll by measuring the transmittance of the leaf in the red and near-infrared regions. Using these two transmittances, the meter calculates a numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf (see Figures 1 and 2).

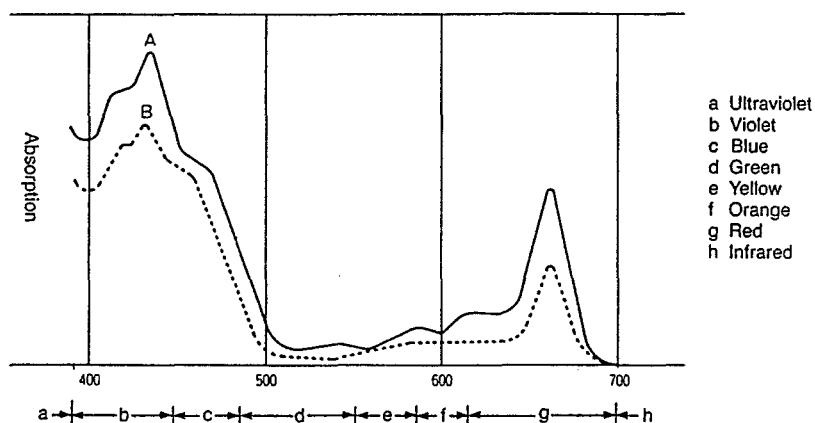


Figure 1 Spectral absorption characteristics of chlorophyll

Figure 1 shows the spectral absorption characteristics of chlorophyll extracted from two leaves using 80% acetone. The chlorophyll content of leaf B is less than that of leaf A. The graph also shows that the peak absorption areas of chlorophyll are in the blue and red regions, with low absorptions in the green region and almost no absorption in the infrared region. Based on this, the wavelength ranges chosen to be used for measurement are the red area (where absorption is high and unaffected by carotene) and the infrared area (where absorption is extremely low).

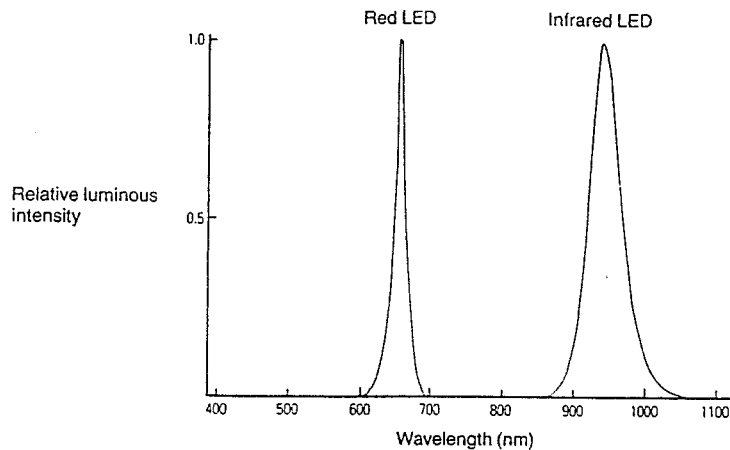


Figure 2 : LED luminous intensity characteristics

Two LEDs, a red LED (peak wavelength: approx. 650nm) and an infrared LED (peak wavelength: approx. 940nm), provide illumination.

The calculation of the chlorophyll meter reading is performed according to the following procedure.

1. During calibration, the two LEDs emit light sequentially without any sample in the sample slot. The received light is converted into electrical signals and the ratio of their intensities is calculated.
2. After a sample has been inserted in the measuring head, the two LEDs emit light again. The light transmitted through the leaf strikes the receptor and is converted into electrical signals. Then the ratio of the intensities of the transmitted light is calculated.
3. The values obtained in steps 1 and 2 are processed to calculate the chlorophyll meter reading, which corresponds to the amount of chlorophyll present in the sample leaf.

3.2 Nitrate Meter and test strips

Whilst evaluating the chlorophyll meter, the opportunity was taken to evaluate a sap nitrate meter which also gives indications of the N status of plant material.

The nitrate meter used test strips which changed colour according to the concentration of nitrate present in a sample of sap which was extracted from the mid-ribs of cane leaves. A test strip is placed in the meter and calibrated. The strip is then dipped in the sap and then placed back into the meter and a reading in mg/l of nitrate obtained.

4. Research Methodology

This project was conducted on experimental sites which had the following characteristics:

- (1) Featured measurements of cane and sugar yield over treatments that varied in N supply.
- (2) Had weather and irrigation amounts carefully monitored.
- (3) Were free from growth limitations other than nitrogen, or had the presence of such limitations well documented.

Sites that were selected to evaluate the chlorophyll meter were ;

- (1) CSC4S sites at Macknade.
- (2) CSC7S site at Schulte farm in Bundaberg.
- (3) BSES trickle irrigation sites in Bundaberg.
- (4) Sludge and N fertiliser trials at Harwood.

The main experimental activity was centred around Bundaberg where Schulte farm was sampled at approximately 2 month intervals and the BSES plots (Coach Rd) at 1 month intervals during the growth period. Three samplings were made at Macknade and one at Harwood. Full details of experimental sites and treatments are provided in Table 1.

Table 1. Details of experiments used in the chlorophyll meter project.

Site	SRDC Project	Organisation	Scientists	Cultivar	Occasions	Treatments
Schulte	CSC7s	CSIRO-TCP	B.Keating V. Catchpoole	CP51/21	5	N0 in 1992/93 + 0 kg N/ha in 1993/94 N1 in 1992/93 + 0 " " N2 in 1992/93 + 0 " " N3 in 1992/93 + 0 " " N0 in 1992/93 + 170 " " N1 in 1992/93 + 170 " " N2 in 1992/93 + 170 " " N3 in 1992/93 + 450 " "
BSES Coach Rd.	BS64s	BSES	R. Ridge G. Kingston	Q136	6	1. Flood - 180kgN/ha(conventional fert.) 2. Trickle - 180kgN/ha(" " " 3. Trickle - 90kgN/ha (fertigation) 4. Trickle - 135kgN/ha (" ") 5. Trickle - 180kgN/ha (" ")
Macknade	CSC4s	CSIRO-TCP / CSR	R. Muchow M. Robertson A. Wood	Q117	3	N1 0 kgN/ha N2 55 kgN/ha N3 357++ kgN/ha
Harwood	-	NSWA	M. Hughes		1	

For each plot the following procedure was undertaken :

Between the hours of 6 am and noon, 20 leaves were collected of each of the leaf classes listed below. The chlorophyll measurements were done on dry leaves as it seems that moisture on the leaf surface has a slight affect on the internal calibration of the meter.

- (1) Top visible dewlap
- (2) The third leaf below class 1
- (3) The oldest leaf with > 75% green tissue

These leaves were sampled on 20 stalks per plot which were selected at random but represented the main population of plants. As well as the above, another 5 stalks were selected and all the leaves collected to determine the canopy nitrogen content.

Of the 20 leaves of each class, 15 were selected at random for chlorophyll measurements. Following the procedures outlined by Farquhar and Lee (1962), the midpoints of each leaf was located by bending the tip back onto the other end and a chlorophyll measurement done on each of the leaf blades at this point. Where the

blade was not wide enough, the section was separated from the mid-rib. This gave 30 measurements which is the maximum the chlorophyll meter can store in memory. The mean was then displayed for that class and noted on the data sheet. Leaf areas were measured on all three leaf class samples and the bulk sample of leaves from the whole canopy. Leaves were subsequently dried at 60 °C and processed for chemical analyses at CSIRO, Brisbane for nitrogen, phosphorus and potassium.

The remaining five leaves had their mid-ribs separated from the leaf blades. The mid-ribs were then cut into sections from 3-5 mm in length, bruised in a mortar and pestle, and then placed in a garlic press and crushed until sufficient sap was obtained. The sap was drawn into a plastic transfer pipette and tested for N content using a sap nitrate meter. Three tests were done on each sap sample and the mean obtained.

The samples were analysed for nitrogen and phosphorus simultaneously on a Technicon AutoAnalyser. Nitrogen was determined by an adaption of the indophenol blue colour reaction and phosphorus by development of molybdenum blue colour. Potassium was determined on a Varian AA-5 atomic absorption spectrophotometer.

4. Results

Cane and sugar yields in relation to Nitrogen treatments

All experiments were established for reasons other than this project and hence a full description of crop performance in relation to nitrogen treatments is beyond the scope of this report. However, the extent to which leaf nitrogen or leaf chlorophyll was correlated to yield response is relevant and a summary of crop yield data is presented in Table 2.

The Macknade crop was a first ratoon of Q117, ratooned 18/8/93. The BSES crop was a first ratoon of Q136. The Schulte crop was a third ratoon of CP51/21, ratooned 2/8/93.

Table 2. Summary of nitrogen treatments, agronomic details and crop yield data for the four experiments examined in this study.

Site	Treatment	Cane Yield (t ha)	CCS (%)	Sucrose (t ha)	Relative Yield (%)
Macknade	0 kg N ha	73	17.3	12.6	53
	55 kg N ha	118	17.8	21.0	88
	357++ kg N ha	167	14.3	23.9	100
BSES	Conventional 180	111	13.2	14.6	95
	Trickle 180	118	13.1	15.4	100
	Fertigate 90	95	13.0	12.3	80
	Fertigate 135	113	12.9	14.5	94
	Fertigate 180	108	12.4	13.4	87
Schulte	N0 + 0	62	14.6	9.1	66
	N1 + 0	78	15.0	11.7	85
	N2 + 0	85	13.6	11.5	84
	N3 + 0	87	13.3	11.5	84
	N0 + 170	93	14.8	13.7	100
	N1 + 170	95	14.4	13.6	100
	N2 + 170	94	12.9	12.2	89
	N3 + 450	91	13.1	11.9	87

First figure refers to N rate in 1992/93 : N0=zero, N1=170, N2=340, N3=170+ 5xNH₄SO₄ side-dressings @ 75 kg N ha⁻¹. Second figure refers to N rate in 1993/94 in kg N ha⁻¹

In summary, a strong N response was recorded at Macknade, with sugar yield reduced to about half the potential in a treatment that received zero fertiliser N in the ratoon crop that was monitored in 1993/94. This treatment received reduced N (approximately 50kg N ha⁻¹ in the plant crop the previous year).

A 20% yield reduction was recorded at the BSES trickle site when N rate over the season was reduced to 90 kg N ha⁻¹ compared to 180 kg N ha⁻¹ applied conventionally.

Sugar yield at the Schulte site was reduced by approximately one third when N fertiliser was omitted for two consecutive seasons, compared to crops receiving the conventional rate of 170 kg N ha⁻¹ in both seasons. Smaller reductions of the order of 15% were recorded for crops receiving conventional or higher rates in 1992/93 and no N fertiliser for the first time in monitored crop in 1993/94. The small decline in sugar yield in the crops receiving excess N application was thought to be due to the effect of NH₄SO₄ side-dressings used in this treatment in lowering pH at this site.

The monitoring at Harwood took place on only one occasion and no relationships with crop yield could be established for this site.

Effect of leaf class on the meter vs leaf N% relationship

The chlorophyll meter readings were significantly correlated with leaf nitrogen percent, but relationships differed significantly between leaf classes (Figure 3 a,b,c,d)

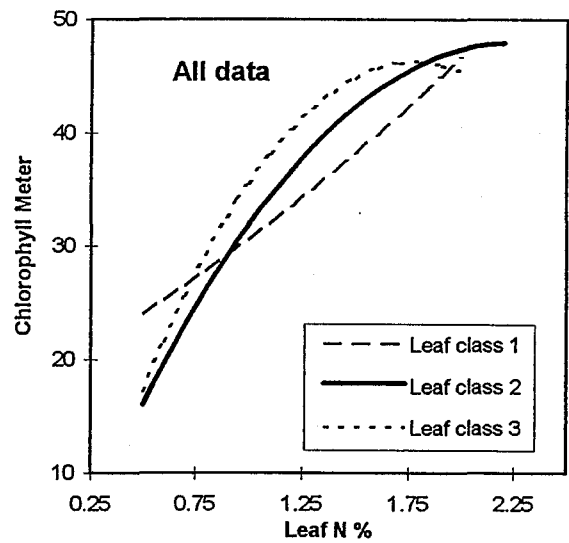
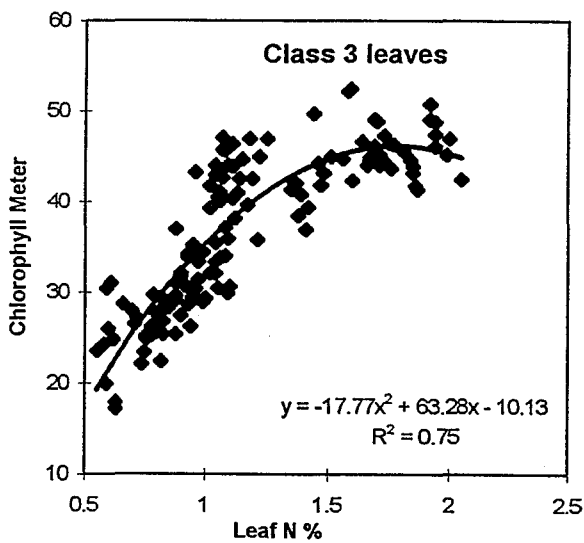
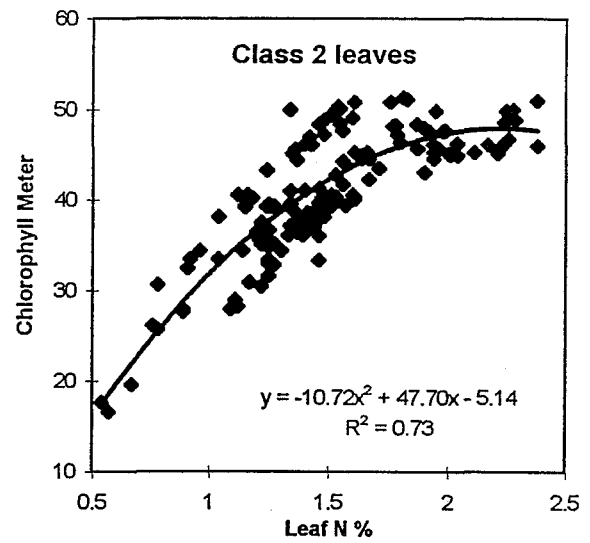
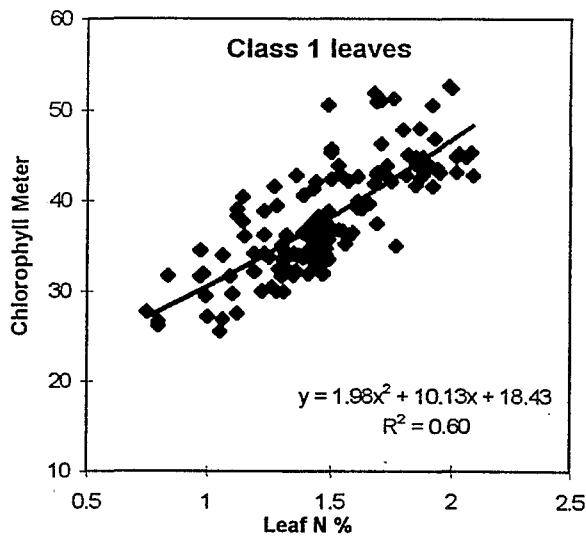


Figure 3. Relationship between chlorophyll meter reading and leaf N % for (a) Class 1 leaves (b) Class 2 leaves (c) Class 3 leaves (d) Comparison of three leaf classes.

Leaf classes 2 and 3 provided the best correlations between leaf N% and chlorophyll meter reading. The chlorophyll meter reading increased with leaf age, for the same leaf N percent (Figure 3d). Leaf class 2 (i.e., three leaves below the uppermost fully expanded leaf) was selected for closer analysis in this report on the basis of;

- the good correlation between chlorophyll meter reading and leaf N %
- the correspondence with the third leaf index used traditionally in the industry.

Unless otherwise stated, the remainder of the data presented in this report will be from Leaf Class 2.

Relationships between meter reading and leaf N determined for each site

Chlorophyll meter readings were correlated with leaf N percent at all sites studied (Figure 4 a,b,c,d). Coefficients of determination ranged from 0.73 to 0.92. Data from one measurement occasion (21/12/93) at the Schulte site were omitted from these regressions on account of spurious results thought to have been associated with extensive leaf rust infection on this crop at that time.

No significant difference was detectable between the chlorophyll meter vs leaf N% relationships determined for the Macknade and Schulte sites (Figure 5). The equivalent relationship from the BSES site was significantly different, exhibiting lower chlorophyll readings for the same leaf nitrogen percent (Figure 5). The relationship determined for the limited data collected at Harwood covered a much lower leaf nitrogen range, but was consistent with the relationships developed at higher leaf N at the Macknade and Schulte sites.

Chlorophyll meter vs specific leaf N relationships

Leaves sampled did differ in specific leaf area (i.e., leaf area in cm^2 per leaf weight in g) over time and sites (Figure 6). Specific leaf areas were generally in the range 85 to 95 $\text{cm}^2 \text{g}^{-1}$. Values from the BSES site were generally higher (i.e. 110 to 120 $\text{cm}^2 \text{g}^{-1}$) while the one year old crop at coming out of a winter at Harwood exhibited very low SLA. These differences infer differences in "leaf thickness" although the weight of the mid-rib does complicate the interpretation. Expressing leaf nitrogen on a per unit leaf area basis rather than a per unit weight basis has been shown in other situations to relate better to leaf physiological function (Muchow and Davis 1988). Hence, the chlorophyll meter vs leaf nitrogen relationships were re-examined with specific leaf nitrogen (SLN) replacing leaf nitrogen percent as the dependant variable. Overall, the goodness of fit was reduced by this step, largely because of the impact on the data pairs from Harwood. The use of specific leaf nitrogen as the dependant variable did, however, remove much of the site differences between the Macknade, Schulte and BSES sites (Figure 7).

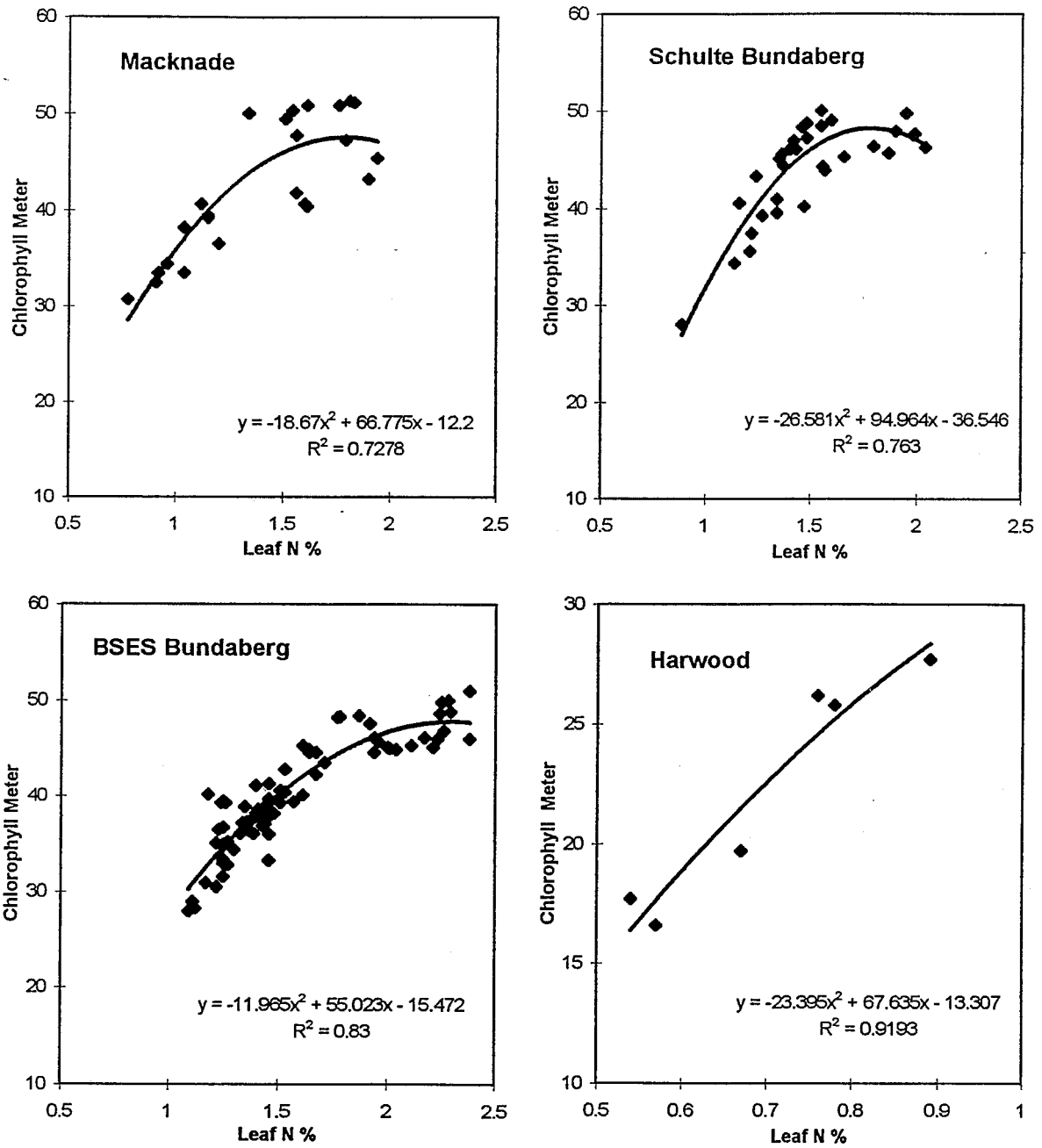


Figure 4. Relationship between chlorophyll meter reading and leaf N % determined for each site (a) Macknade (b) Schulte Bundaberg (c) BSES Bundaberg (d) Harwood.

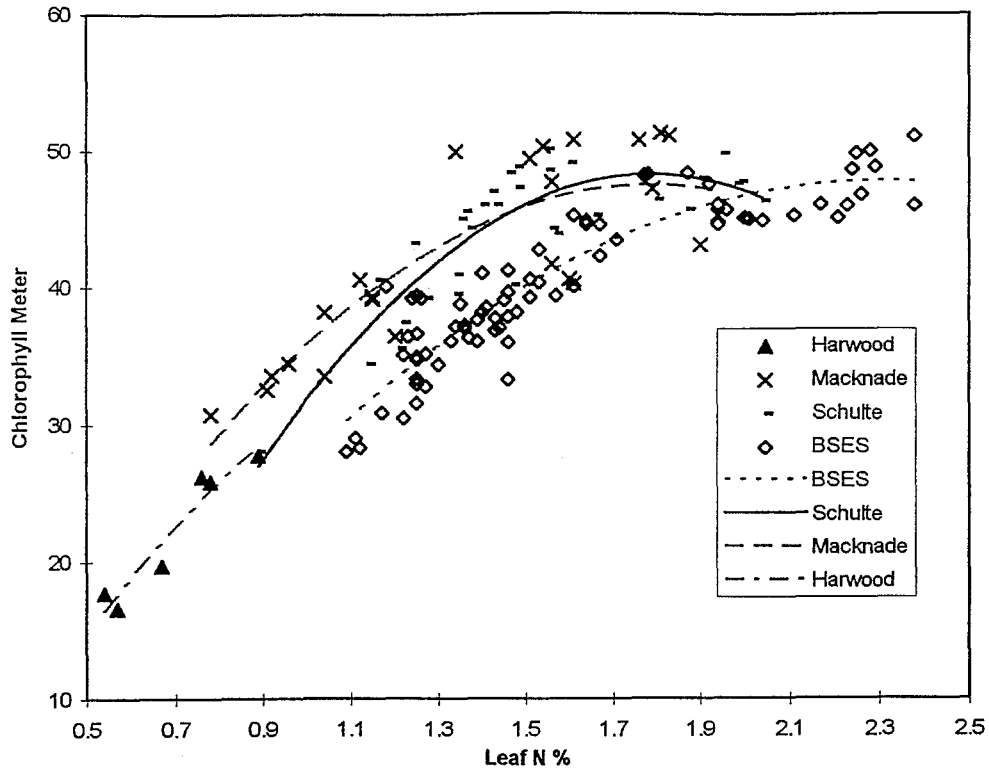


Figure 5. Relationship between chlorophyll meter reading and leaf N % for all sites studied (a) Macknade (b) Schulte Bundaberg (c) BSES Bundaberg (d) Harwood.

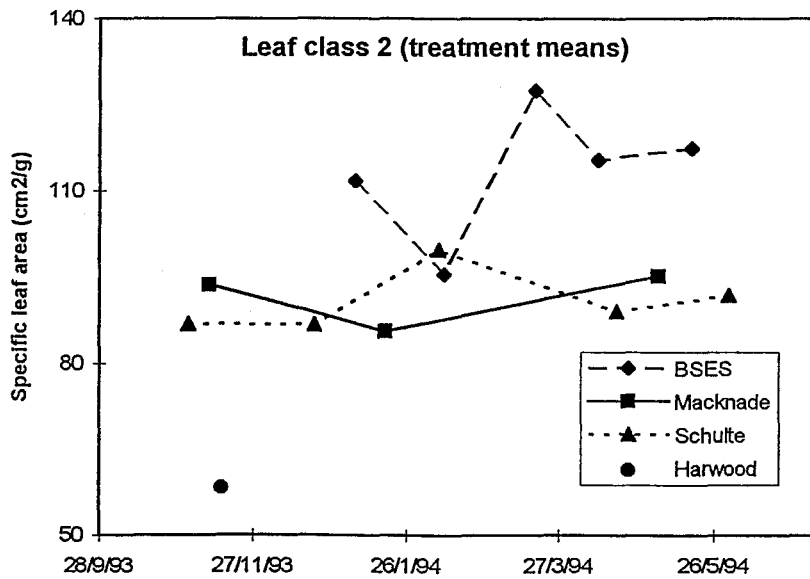


Figure 6. Relationship between specific leaf area (sq cm leaf area per gram leaf dry weight) and time of year for four locations. (Values shown are the treatment means).

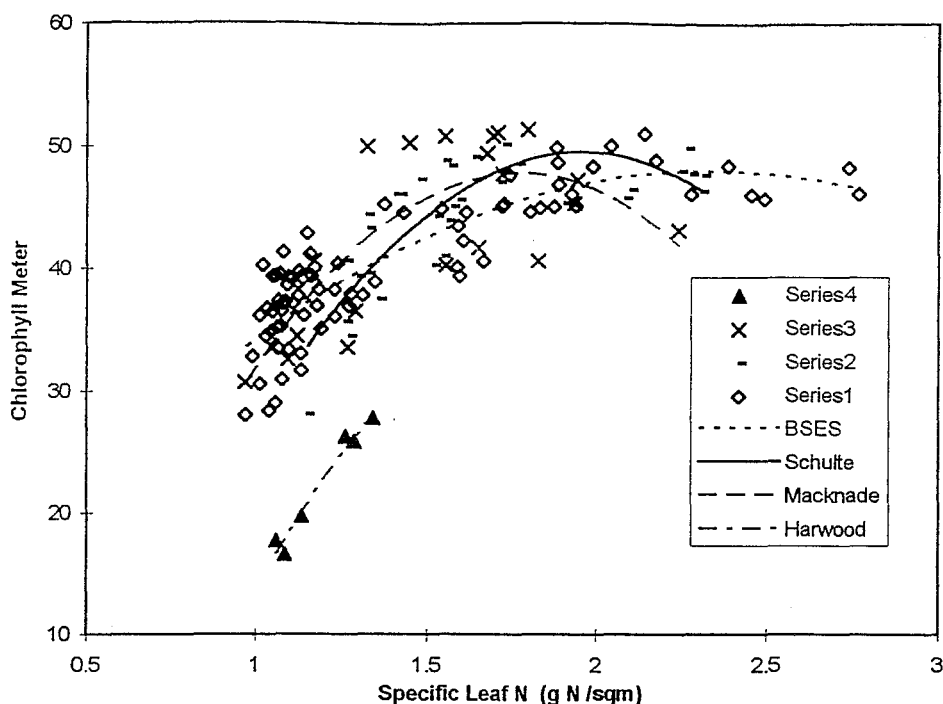


Figure 7. Relationship between chlorophyll meter reading and specific leaf N (g N m^{-2} of leaf area) for four sites.

Changes in N content with time and relationship with N treatments.

Leaf nitrogen percent of all leaf classes generally fell with time (Figure 8a,b,c), a response that was most marked at the Schulte and BSES sites (Figure 8b,c)). An unusual increase in leaf N percent was noted late in the season at Macknade (Figure 8a), and this may have been related to low N treatments assessing significant amounts of nitrogen that was transferred from the high N treatment via the water table at this site.

The equivalent trends in chlorophyll meter reading are presented in Figure 9. Because the chlorophyll vs leaf N % relationship is curvi-linear, leaf nitrogen percent has to drop below some critical value before this registers as a drop in chlorophyll meter reading. Hence, the shape of the temporal trends in Figure 9 are different to those shown in Figure 8.

In general, lowest leaf N percent and chlorophyll meter readings were obtained from the treatment that received the lowest N rate.

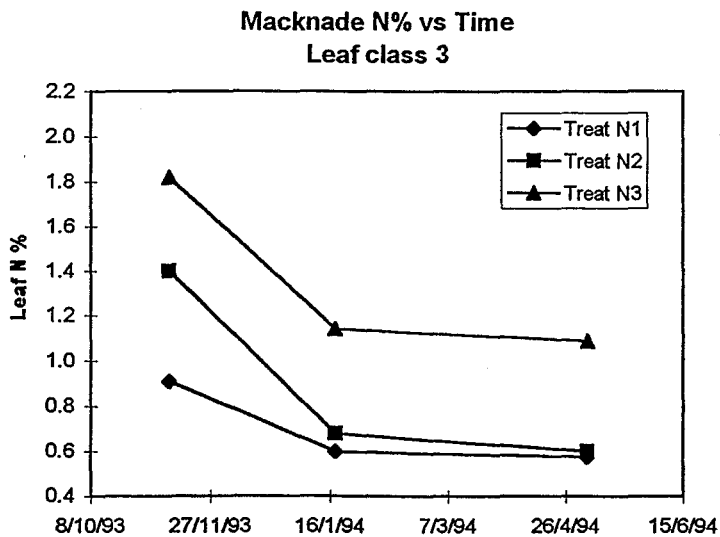
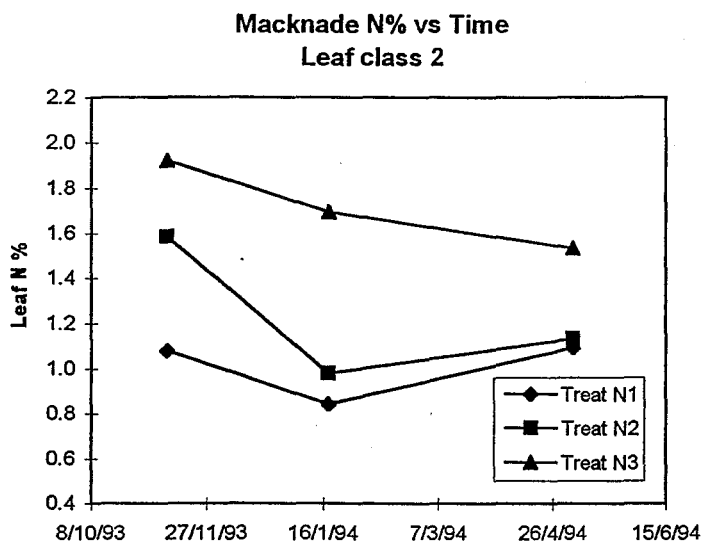
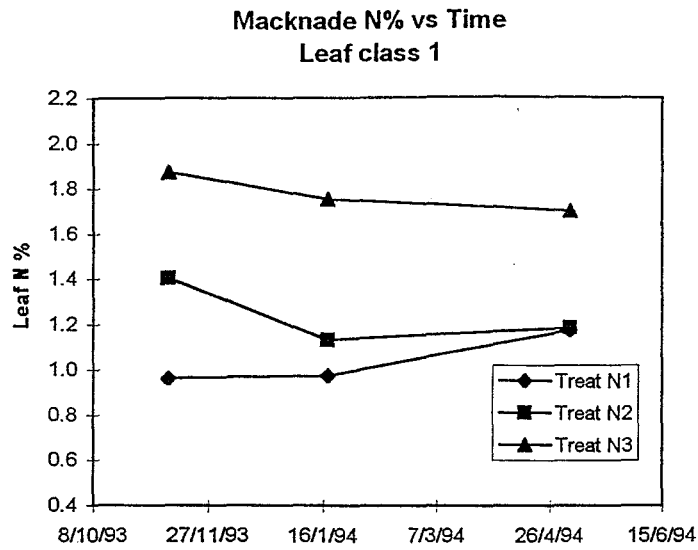


Figure 8a. Temporal trends in leaf N % for three leaf classes and various N treatments at Macknade.

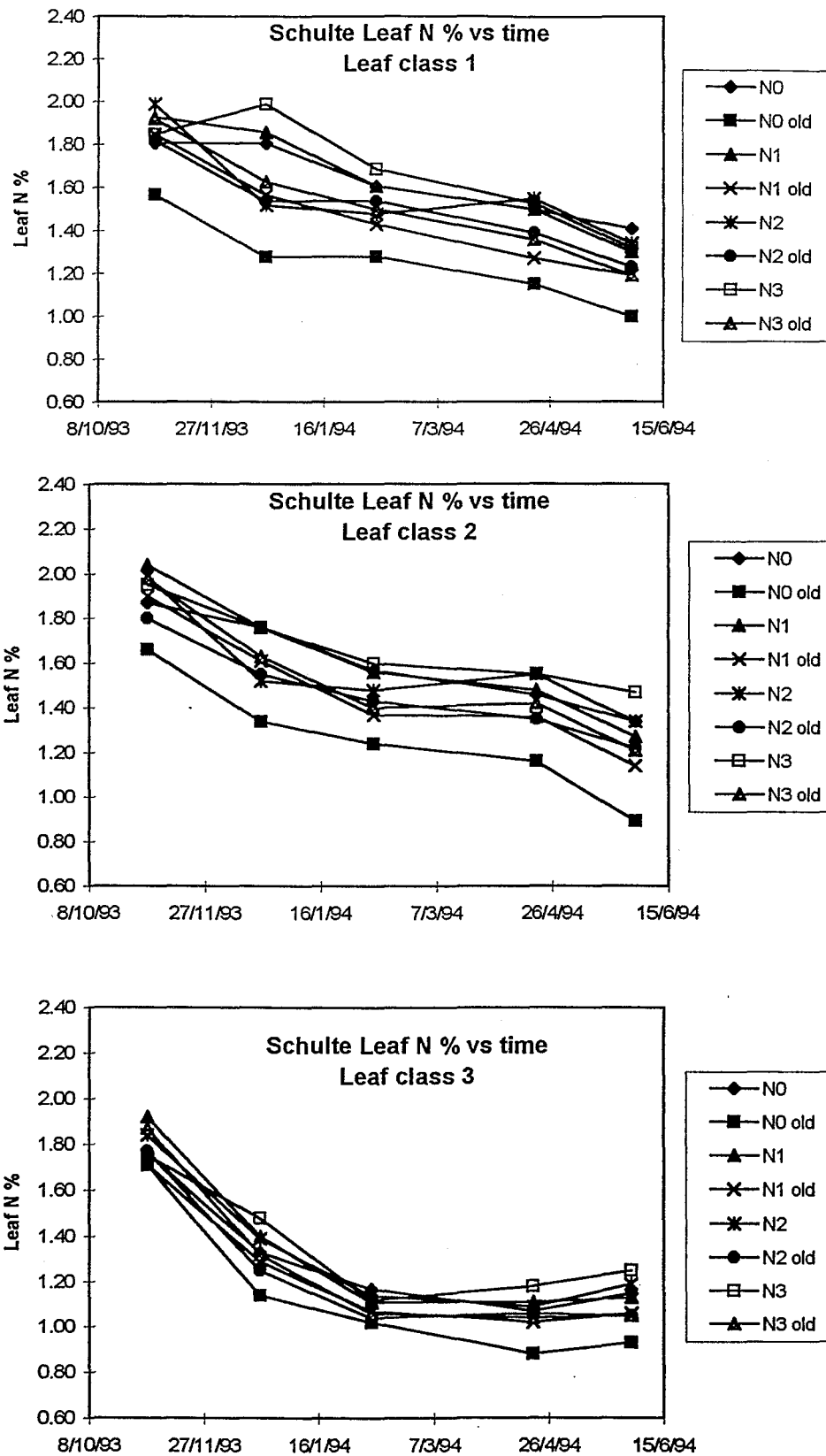


Figure 8b Temporal trends in leaf N % for three leaf classes and various N treatments at the Schulte site, Bundaberg. N rates in kg N ha^{-1} for 92/93 + 93/94 were ; N0 = 0+170, N0 old = 0+0, N1 = 170+170, N1 old = 170+0, N2 = 340+170, N2 old = 340+0, N3 = 450+170, N3 old = 450+0.

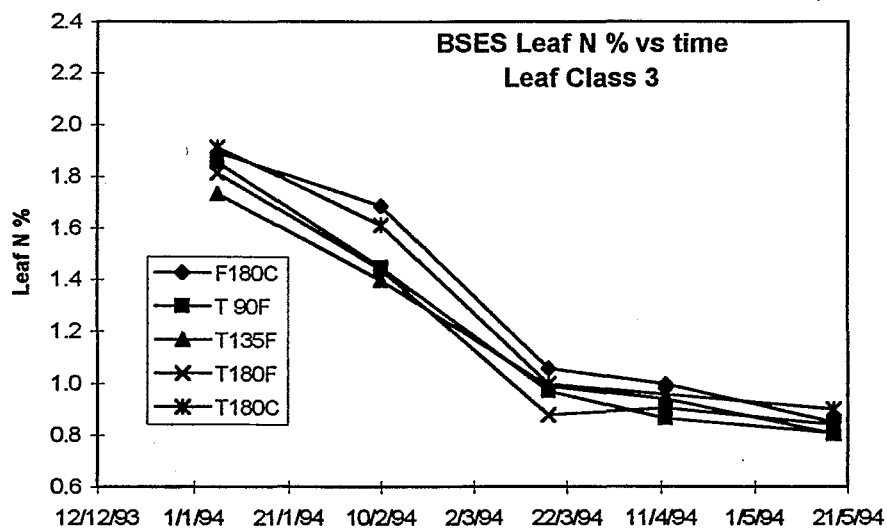
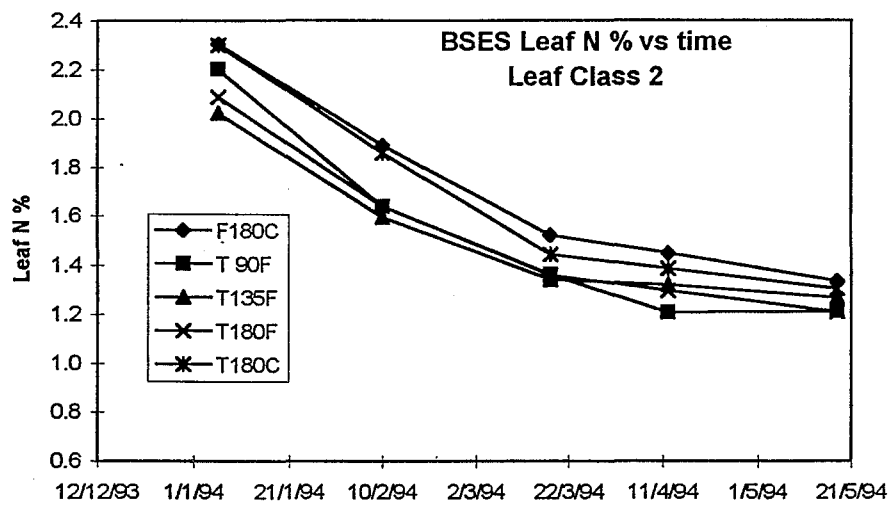
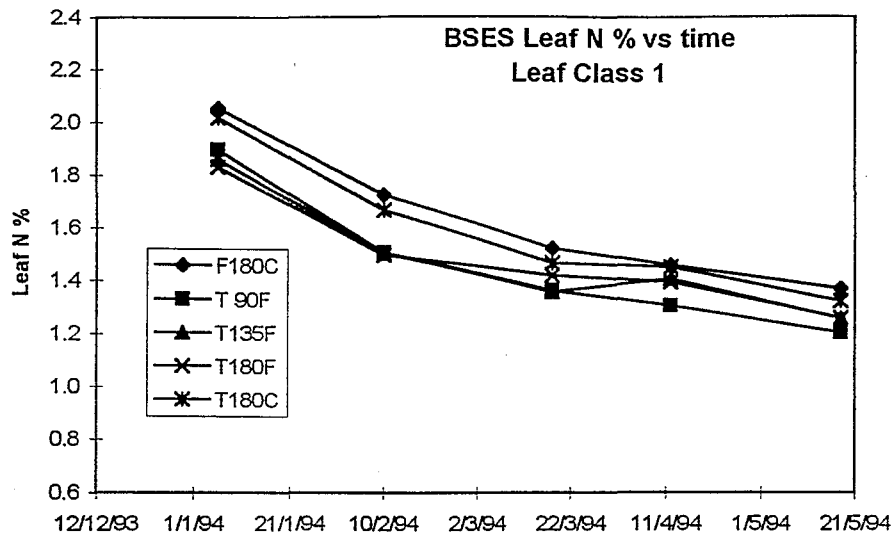
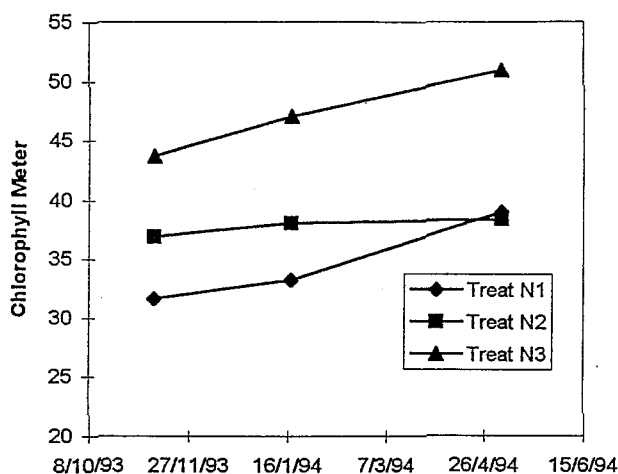
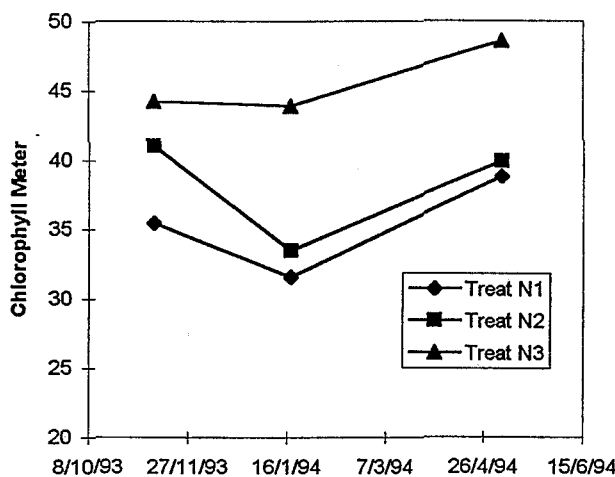


Figure 8c. Temporal trends in leaf N % for three leaf classes and various N treatments at the BSES site, Bundaberg. Legend = Irrigation method (F = flood, T = trickle) / Nrate (in kg N ha⁻¹) / Fertilisation method (C = solid single application, F= fertigation over 4 times)

Macknade Chlorophyll Meter vs Time, Leaf class 1



Macknade Chlorophyll Meter vs Time, Leaf class 2



Macknade Chlorophyll Meter vs Time, Leaf class 3

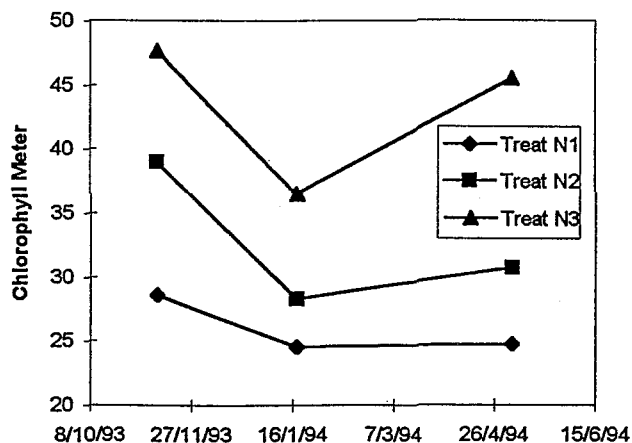


Figure 9a. Temporal trends in the chlorophyll meter reading for three leaf classes and various N treatments at Macknade.

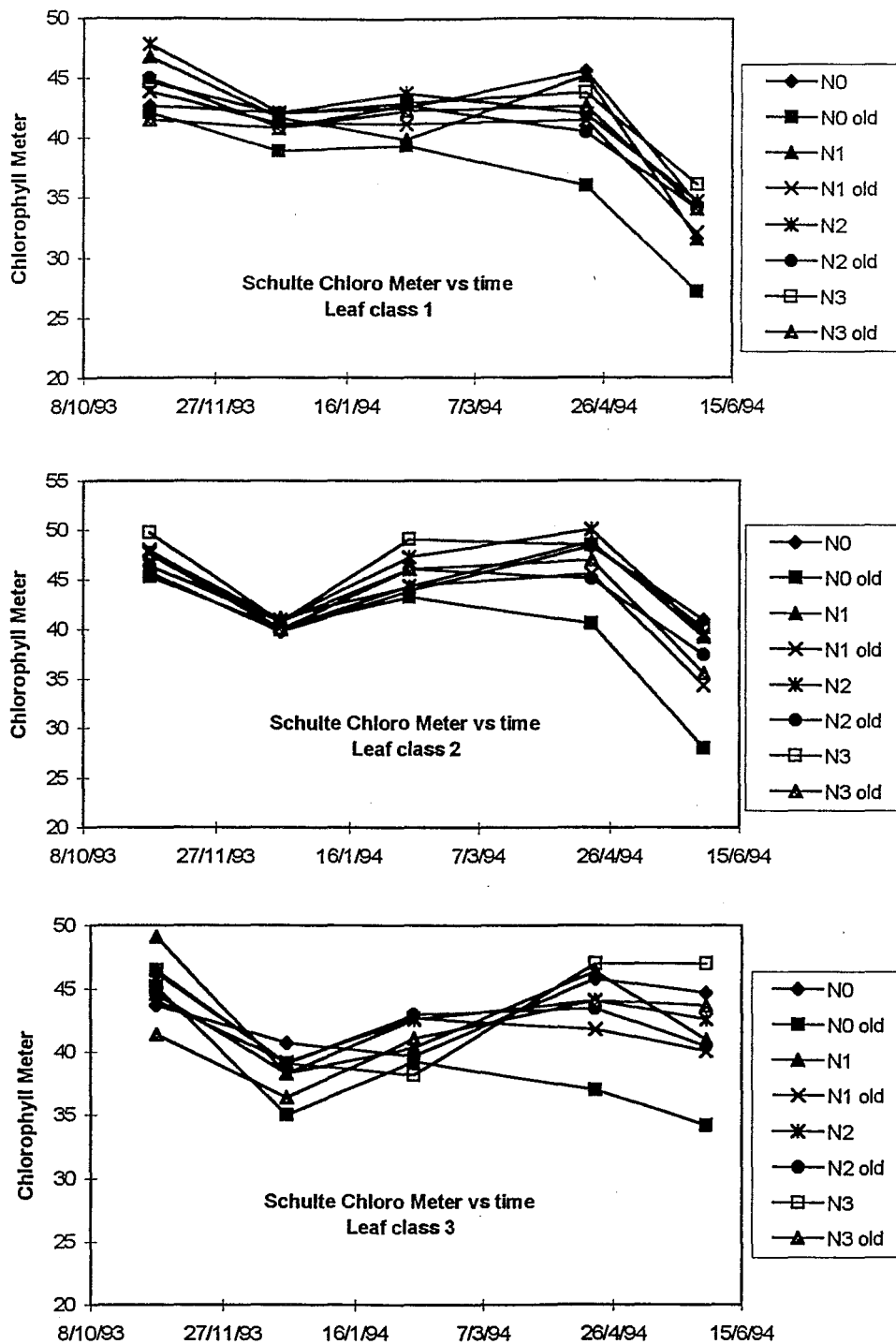


Figure 9b Temporal trends in the chlorophyll meter reading for three leaf classes, and various N treatments at the Schulte site, Bundaberg. N rates in kg N ha⁻¹ for 92/93 + 93/94 were ; N0 = 0+170, N0 old = 0+0, N1 = 170+170, N1 old = 170+0, N2 = 340+170, N2 old = 340+0, N3 = 450+170, N3 old = 450+0.

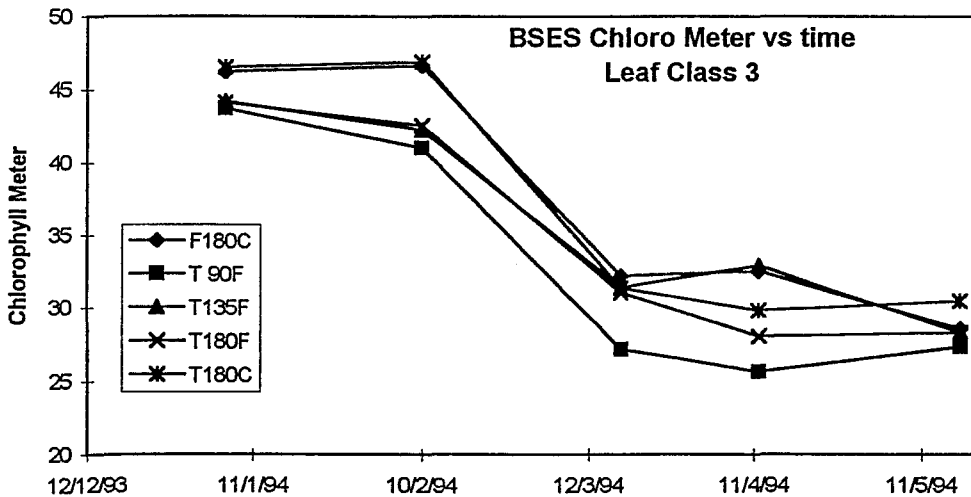
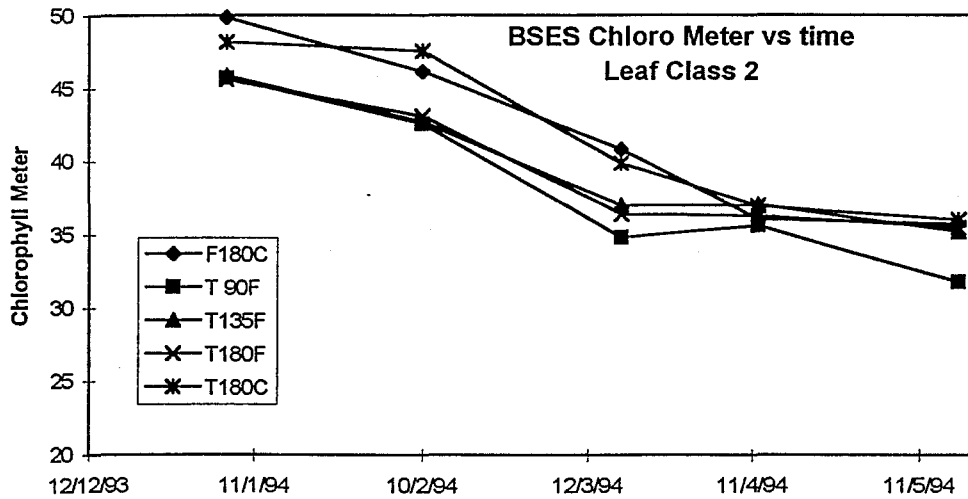
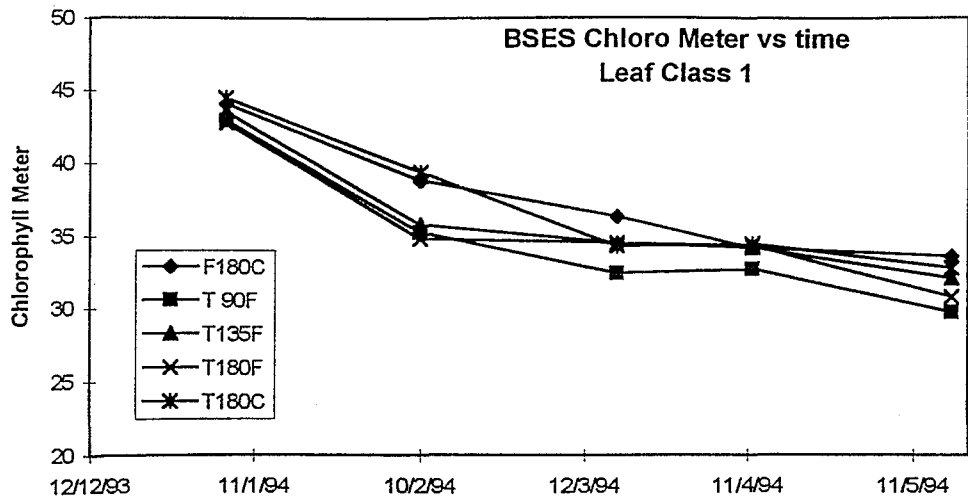


Figure 9c. Temporal trends in chlorophyll meter reading for three leaf classes and various N treatments at the BSES site, Bundaberg. Legend = Irrigation method (F = flood, T = trickle) / Nrate (in kg N ha⁻¹) / Fertilisation method (C = solid single application, F= fertigation over 4 times)

Trends in leaf N % and meter reading within the canopy

Trends in leaf nitrogen percent down the canopy were small, but the oldest leaf class (Class 3) generally exhibited the lowest leaf N %. A typical dataset is shown in Figure 10 for Macknade.

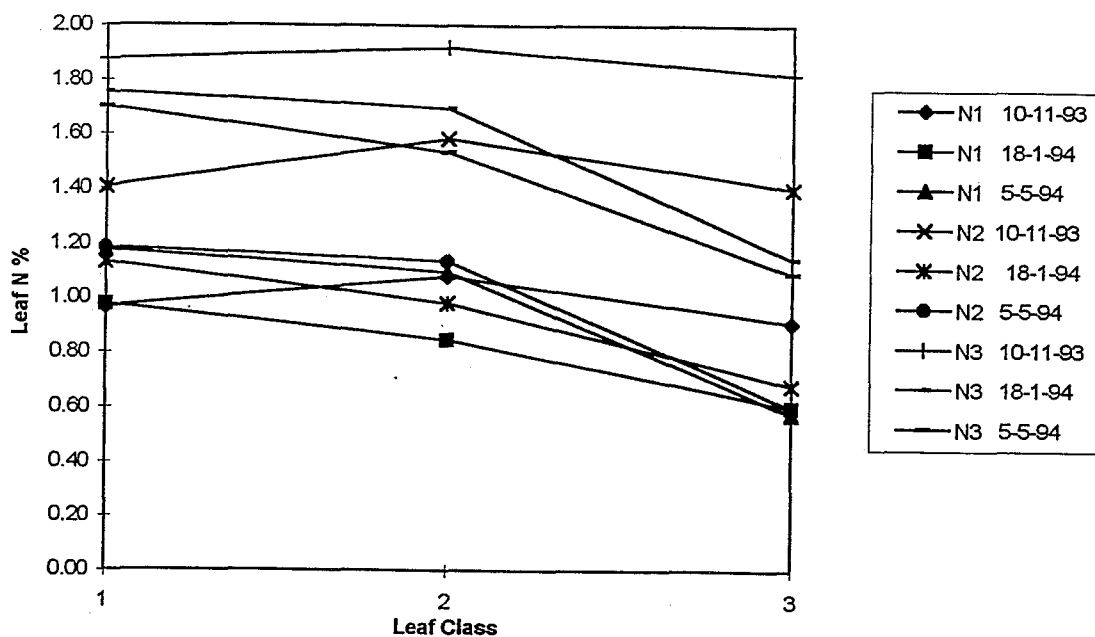


Figure 10. Variation in leaf N % with leaf class (Class 1 is high in the canopy, Class 3 is low in the canopy) for three N treatments and various dates (see Table 1) at Macknade.

Correlations between Leaf N % and canopy N %

The data collected in the study allow canopy N percent to be correlated with the N % of the three leaf classes monitored. Leaf classes 1 and 2 contain leaves with an N percent higher than the bulk canopy, while the N% of class 3 leaves is lower (Figure 11). The mean of leaf classes 1, 2 and 3 approximates the canopy N percent.

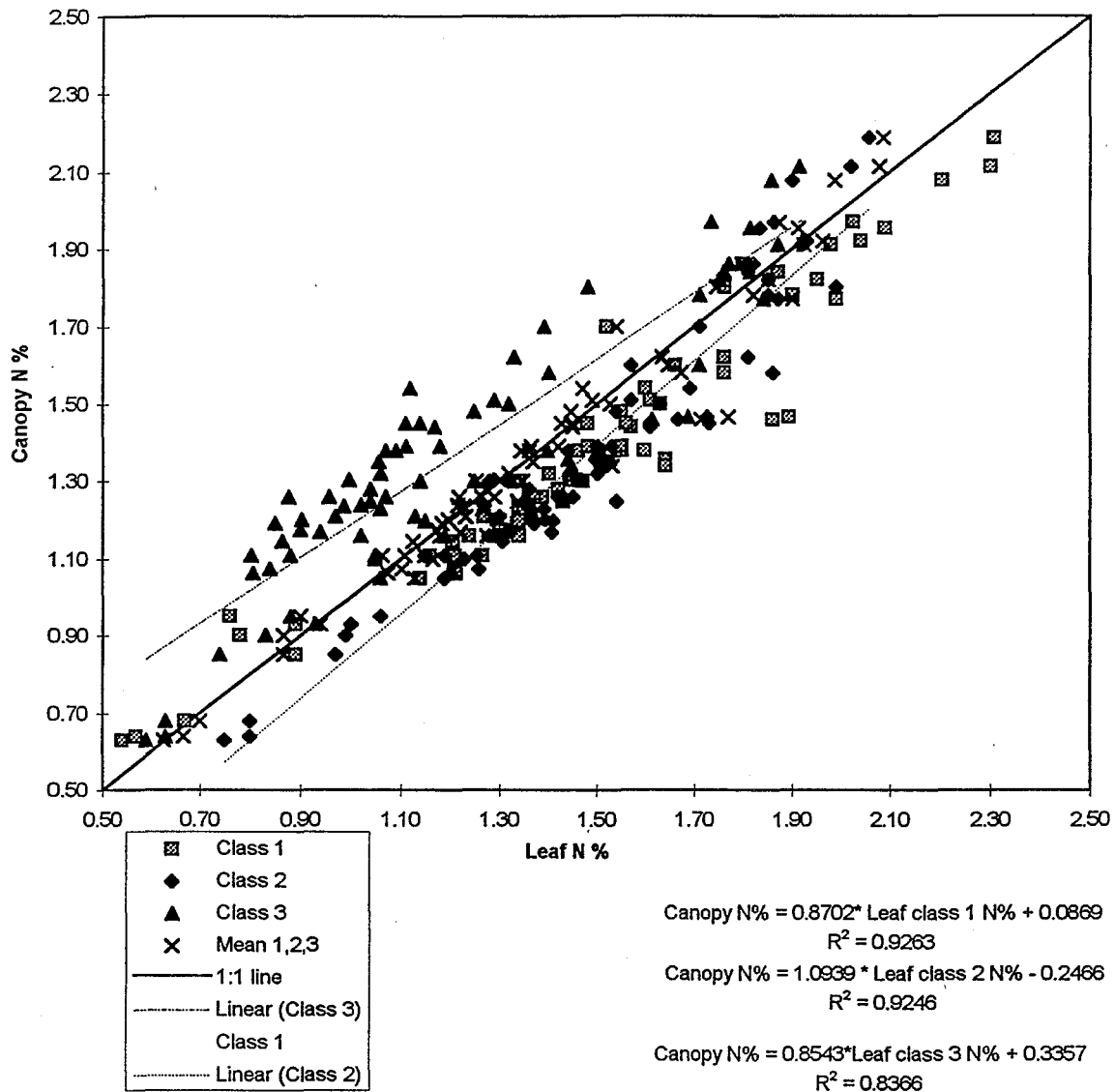


Figure 11. Relationship between N % determined on the bulk canopy and N % of individual leaf classes.

Relationship between chlorophyll meter or leaf N % and sugar yield

The various N fertiliser treatments resulted in reductions in final sugar yields over range from 0 to 50%. The majority of N limited treatments reduced sugar yields by 10 to 20 %. There was some evidence that these reductions in sugar yield could have been detected with measurements of either leaf N% (Figure 12a) or the chlorophyll meter (Figure 12b), although variability in the data make them far from convincing. All the data shown in Figure 12 were collected over the November to March period. There is evidence that a 5 to 10 % reductions in chlorophyll meter readings early in crop development, relative to some high N treatment, were associated with reductions in final sugar yield of the order of 10 to 20 %.

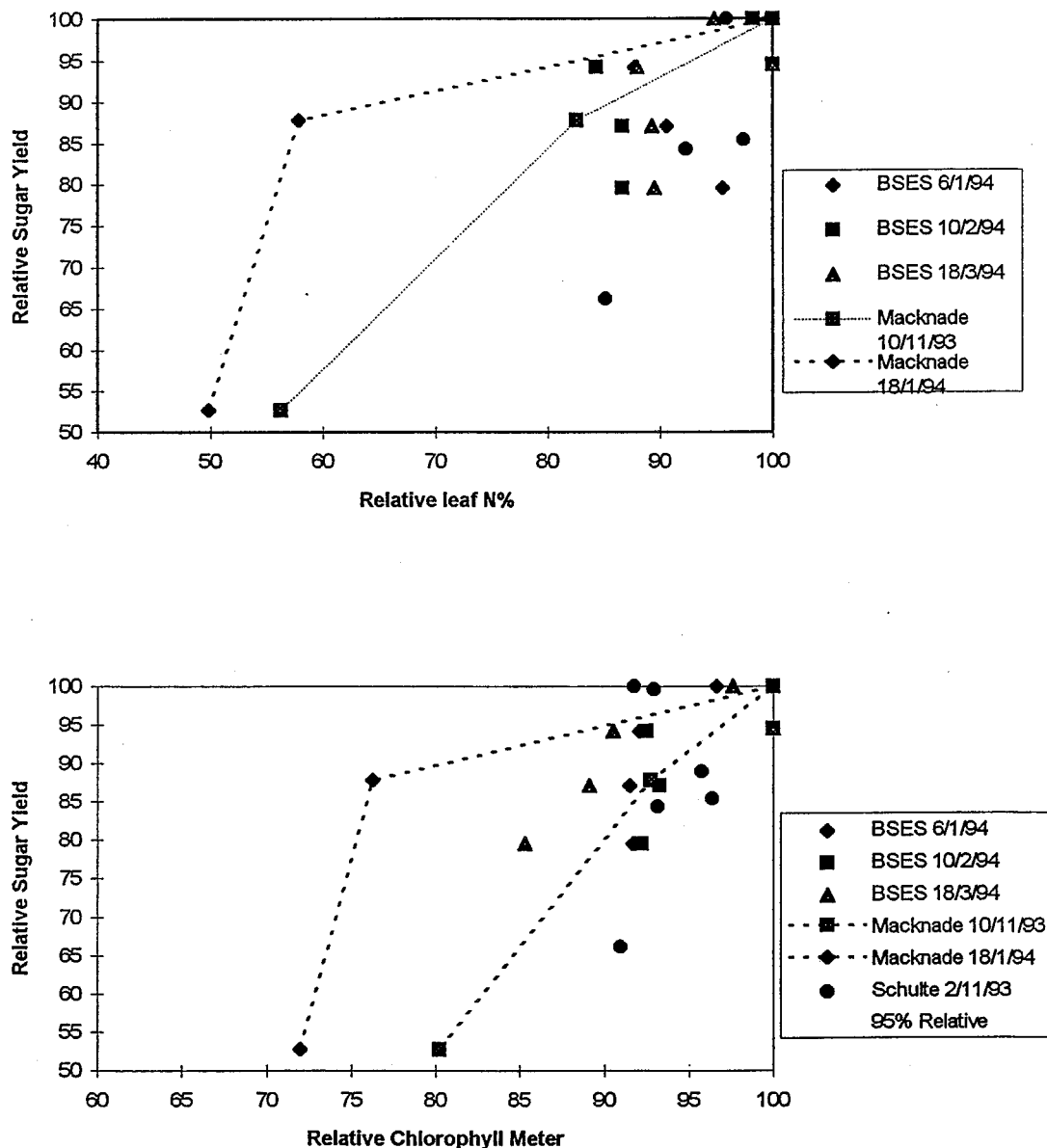


Figure 12. Relationship between relative final sugar yield (relative to the highest yielding, "high N" treatment) and relative (a) leaf N % and (b) chlorophyll meter reading. Both measures of leaf N status are expressed relative to the highest N status treatment for each individual measurement occasion.

Overall relationship between sap nitrate and leaf N%

Sap nitrate data were generally not well correlated with leaf N% (Figure 13). Low N treatments tended to have lower sap nitrate (Figure 14) but data were highly variable and all values were generally low (< 10 ppm) compared to the values in excess of 5000 ppm recorded in some vegetable crops (Olsen and Lyons 1994)

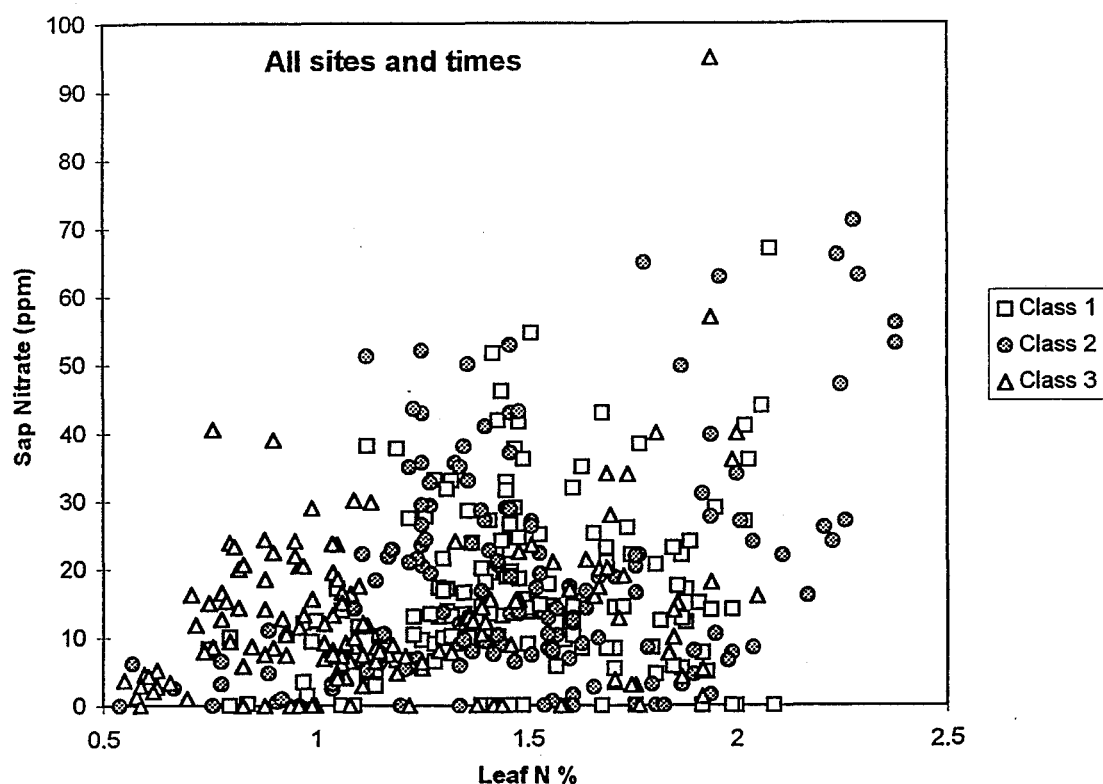


Figure 13. Relationship between sap nitrate concentration for the mid-ribs of three sugarcane leaf classes and whole leaf N%. Data come from sites, treatments and occasions as detailed in Table 1.

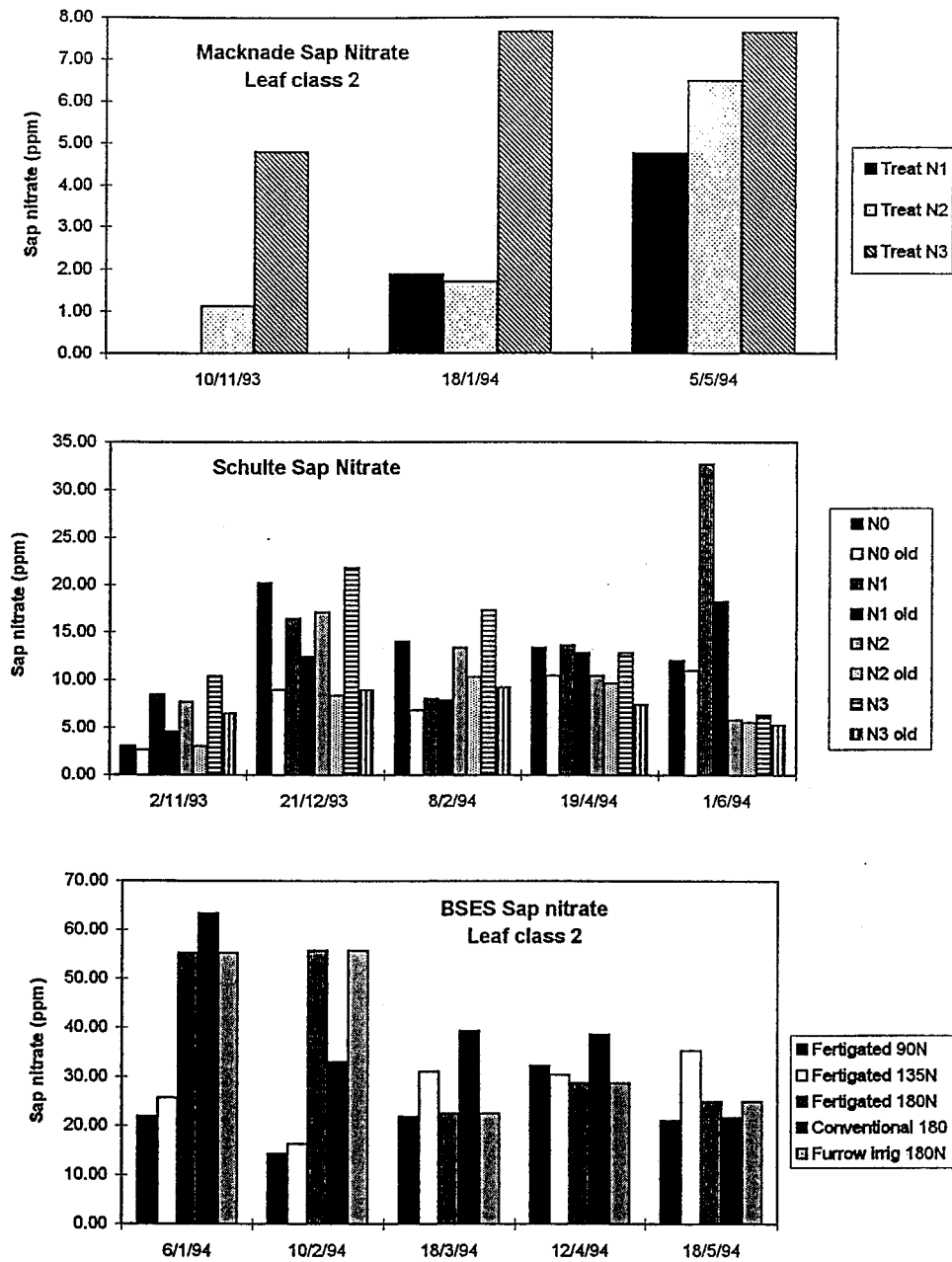


Figure 14. Temporal variation in sap nitrate for mid-ribs from Class 2 leaves from a range of N treatments at (a) Macknade, (b) Schulte Bundaberg, and (c) BSES Bundaberg. (Sap nitrate of zero was recorded in treatment N1 at macknade on 10/11/93.)

Other nutrients

Potassium and phosphorus were also recorded on leaves analysed for nitrogen. While outside the scope of the current project, data are recorded here for completeness (Appendix 1)

5. General Discussion

The primary objective of this project was to evaluate the leaf chlorophyll meter as a means of assessing the nitrogen status of sugarcane crops. Our objective was to quantify the relationship between leaf nitrogen and chlorophyll meter reading for;

- (1) Crops differing in N status.
- (2) Crops of different growth stage, cultivar and ratoon.
- (3) Crops grown in environments ranging from northern NSW to north Qld.

The project has found that the chlorophyll meter is generally well correlated with leaf N%. Coefficients of determination ranged from 72 to 92 %. The project has also found that leaf class 2 (3 leaves below the uppermost leaf with visible dewlap) provided a suitable index leaf, on the basis of the strength of the correlations, ease of measurement and compatibility with the current local industry practise. The relationships between chlorophyll meter reading and leaf N% are non-linear (Figures 3 and 5). In this report, simple quadratic relationships have been plotted. These can not be extrapolated beyond the range of the data. Functional forms such as the broken stick or Mitcherlich equation could provide a more appropriate representation of the plateau in chlorophyll above meter readings of approximately 45. Direct comparison of the leaf N% data with those reported in the literature (eg., Clements 1953, Baver 1963, Humbert 1968, Wood 1968, Meyer and Wood 1984, Anderson and Bowden 1990) is not simply done because of the great variety of leaf tissues that have been used in these studies. These have included; whole leaves (as in this study), whole leaves less mid-ribs, the middle 20cm of certain leaves and small discs punched from leaf blades, all from a variety of locations in the canopy.

A significantly different relationship was obtained for the BSES site compared to the near-by Schulte site and the distant Macknade site (Figure 5). The crop at the BSES site exhibited a lower chlorophyll meter reading for the same leaf N percent. Cultivar differences are the most plausible explanation, as the environment was generally similar between the BSES and Schulte sites. Expressing leaf N content on a per unit leaf area basis, rather than a leaf weight basis, removed much of the difference between the BSES, Schulte and Macknade relationships, but introduced an off-set for the Harwood data (Figure 7). The overseas experience (Schepers 1994) is that the chlorophyll meter is not reliable as an absolute measure of leaf chlorophyll and N content, because of the effects of factors such as varieties and other stresses on the chlorophyll reading. Rather, its value is to assess the chlorophyll content (and hence N content) of a farmer's bulk crop, relative to some "well fertilised control strip". The variation exhibited in Figure 5 would suggest the same principle would apply for use of the meter on sugarcane crops.

Cultivar differences in leaf N% and chlorophyll meter reading have not been evaluated in this study without some confounding with variation in other factors such as climate or water and N management. Studies elsewhere (Inman-Bamber 1984) and limited Australian experience (R.C. Muchow, pers. comm.) indicate that significant genotypic variation in leaf N% occurs and this will impact at some point on the chlorophyll meter reading. This provides further justification for limiting the use of the chlorophyll meter to the relative comparison of sugarcane crops that vary only in N supply.

Leaf N % generally declines rapidly with crop age (see for example Figure 8a,b,c) and this has created problems in establishing critical values (Gosnell and Long 1971). These too need to be specified in relation to crop age. Variation in “physiological age”, for instance between plant and ratoon crops or between crops grown in different temperature environments, creates difficulties in identifying appropriate critical concentrations. Chlorophyll meter readings also decline with crop age (see Figure 9a,b,c), although the decline is not as marked because of the plateau in chlorophyll meter reading at high leaf N%. This phenomena creates an interpretation problem for absolute chlorophyll meter readings, but is eliminated when using the meter on a relative basis.

The problem of leaf rust encountered on one occasion at least one site, highlights a potential practical limitation to use of the chlorophyll meter test. The test is unreliable on leaves that have been affected by some pest or disease problem.

In comparison with the chlorophyll meter, the sap nitrate test was time consuming and cumbersome. Sap nitrate in sugarcane did not correlate well with leaf nitrogen, although the relative differences between treatments was roughly related nitrogen treatments. This is not surprising as the sap nitrate test reflects nitrate in the xylem flow today, while leaf N% reflects more of the nitrogen history of leaves. The nitrate levels in the cane sap were very low (generally < 20 ppm) compared to the values reported for some horticultural crops where the test is routinely used (eg > 5000 ppm for capsicum, Olsen and Lyons 1994). This we presume reflects the finding that sugarcane predominantly reduces nitrate in the roots and transports nitrogen as amino acids such as asparagine (Ian Biggs, pers. comm.).

6. Implications and Recommendations

This preliminary project has shown that the chlorophyll meter provides a very simple and rapid means by which either farmers or researchers can assess relative leaf nitrogen status, over the range where growth is most affected by N supply.

The meter would appear to be only really of benefit where ;

- Previously undiagnosed N deficiency that was limiting sugar production existed and corrective action in the form of supplementary fertiliser applications was possible.

- There were strong environmental grounds for reducing N fertilisation rates and the meter assisted in the monitoring of such crops to ensure leaf nitrogen / chlorophyll levels did not drop to levels where production was likely to be affected.

A comprehensive assessment of the practical prospects for use of such a monitoring technique to improve N fertiliser management was beyond the scope of this project. Low leaf N early in the annual growth cycle, as manifested by low chlorophyll meter reading, certainly raises the possibility that N supply will limit sugar yields. High leaf N that is reflected in high chlorophyll meter readings (above approximately 45 in sugarcane) is indicative that N is in adequate (or excess) supply. The sensitivity and reliability of this information, together with the degree to which N fertilisation tactics can be employed to improve management remains uncertain at this point. In general, sugarcane has been shown to be best suited to N fertilisation early in the growth cycle, although the physiological basis for this observation has not been well established (Baver 1963, Wood 1968). The data from the BSES site referred to in this study (BS64s) provides further evidence of this phenomena. Highest yields were obtained from those treatments that received all their N fertiliser (180 kg N ha⁻¹) early (6/12/93) as a solid dressing. The same rate yielded 15% less when split into four doses over the December to March. Hence, information on crop N status beyond December/ January may be of little practical value, if N fertilisers are difficult to apply, and responses in sugar yield are uncertain.

In conclusion, a number of uncertainties still exist with respect to the practical value of the chlorophyll meter. These include ;

- Uncertainty over the possible trade-offs between fresh cane yield and sugar content arising from N fertiliser applications made in "mid-season" (i.e., from December to March)
- Practical problems associated with supplementary N fertiliser applications to cane crops that have grown "out of hand".
- Uncertainties over farmer attitudes to more elaborate N fertiliser management strategies.

It is for these reasons we believe we need to look more broadly at a range of crop and soil based monitoring tools. Soil testing may yet be shown to have a place. Likewise, use of cane stem analysis at the mill is something that is intrinsically attractive and is the subject of a new proposal in 1995/96. We shall continue to use the chlorophyll meter in conjunction with N research on sugarcane and look for opportunities to include it in an integrated "tool kit" that will promote improved N fertiliser management.

7. Intellectual property

There are no issues of special significance in relation to intellectual property and this project. The individual field trials will be reported separately by those responsible (Table 1). The results of the chlorophyll meter study will be reported to the industry at an appropriate time and in a broader context than is possible just now. The capital

item (the chlorophyll meter) will continue to be shared amongst N researchers for use in sugarcane experimentation.

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9. Acknowledgements

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Appendix 1. P and K concentrations in leaves used for chlorophyll meter readings

SITE **SCHULTE**

Phosphorus

	Data			
DATE	Average of P Class 1	Average of P Class 2	Average of P Class 3	Average of P Canopy.
2/11/93	0.23	0.21	0.20	0.21
21/12/93	0.19	0.17	0.13	0.18
8/2/94	0.17	0.15	0.10	0.15
19/4/94	0.15	0.14	0.10	0.14
1/6/94	0.13	0.11	0.09	0.11

SITE **MACKNADE**

Phosphorus

	Data			
DATE	Average of P CL. 1	Average of P CL. 2	Average of P CL.3	Average of P CAN.
10/11/93	0.24	0.25	0.24	*
18/1/94	0.21	0.18	0.18	*
5/5/94	0.19	0.18	0.18	*

SITE **BSES**

	Data			
DATE	Average of P Class 1	Average of P Class 2	Average of P Class 3	Average of P Canopy
6/1/94	0.29	0.29	0.26	0.31
10/2/94	0.23	0.21	0.18	0.20
18/3/94	0.20	0.17	0.11	0.17
12/4/94	0.19	0.15	0.10	0.16
18/5/94	0.18	0.15	0.09	0.15

SITE	SCHULTE
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Potassium

DATE	Data			
	Average of K Class 1	Average of K Class 2	Average of K Class 3	Average of K Canopy
2/11/93	1.14	0.94	0.89	1.08
21/12/93	1.40	1.00	0.81	1.31
8/2/94	1.31	1.00	0.79	1.14
19/4/94	1.31	1.10	0.92	1.29
1/6/94	1.45	1.16	0.92	1.14

SITE	MACKNADE
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Potassium

DATE	Data			
	Average of K Class 1	Average of K Class 2	Average of K Class 3	Average of K Canopy
10/11/93	1.33	1.14	1.02	*
18/1/94	1.17	0.95	0.80	*
5/5/94	1.27	1.06	0.88	*

SITE	BSES
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Potassium

DATE	Data			
	Average of K Class 1	Average of K Class 2	Average of K Class 3	Average of K Canopy
6/1/94	1.70	1.57	1.52	1.78
10/2/94	1.45	1.21	0.95	1.22
18/3/94	1.52	1.25	0.84	1.35
12/4/94	1.47	1.13	0.89	1.30
18/5/94	1.57	1.20	0.89	1.30

Appendix 2. Using a Chlorophyll Meter to Improve N Management.
NebGuide G93-1171-A



Using a Chlorophyll Meter to Improve N Management

Todd A. Peterson, Extension Cropping Systems Specialist; Tracy M. Blackmer, Graduate Research Assistant;
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This NebGuide describes how to use a chlorophyll meter as a tool to improve nitrogen management by detecting nitrogen deficiency and determining the need for additional N fertilizer.

Fertilizer nitrogen (N) is increasingly recognized as the source of nitrate contamination in much of Nebraska's groundwater. Improving the efficiency of fertilizer N use reduces the amount of N that can potentially contaminate water resources. Effective management of fertilizer N is a major challenge for grain crop producers. Many factors that affect its efficiency are beyond a producer's control. Weather, equipment limitations and breakdowns, and availability of labor and fertilizer during critical periods can lead to inadequate N supply to the crop. Fertilizer N is relatively inexpensive, and deficiencies can result in substantial yield reductions. Producers are inclined to manage fertilizer N to minimize the risk of deficiency, which can lead to excessive fertilizer applications. Although they understand fertilizer applied at excessive rates costs money and may lead to contamination of the environment, producers also want assurance that applying less fertilizer N will not reduce crop yields.

Researchers have been looking for ways to increase fertilizer N use efficiency. The use of a soil test to adjust fertilizer N rates for residual nitrate works well under Nebraska conditions and producer acceptance of the practice is increasing. However, the potential exists to "fine-tune" N management decisions during the growing season to react to changing weather and crop conditions.

The concept of using tissue testing to provide an assessment of crop N status is not new. Recent research indicates a close link between leaf chlorophyll content and leaf N content, which makes sense because the majority of leaf N is contained in chlorophyll molecules. The Minolta¹ chlorophyll meter

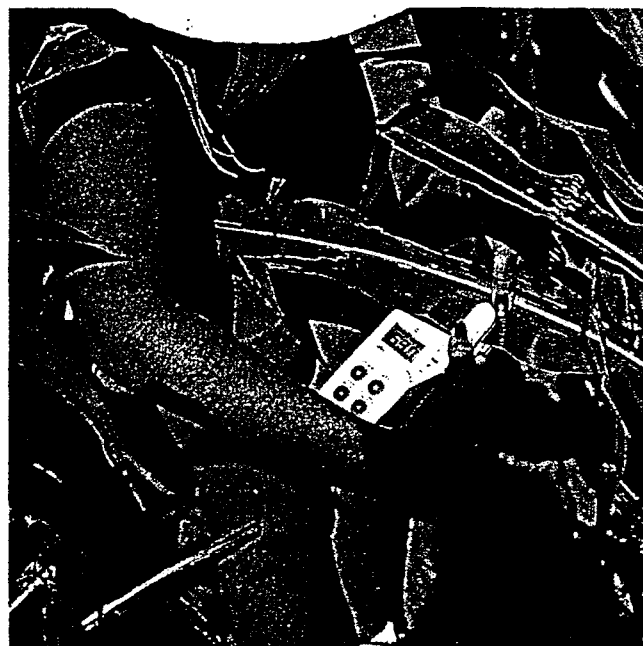


Figure 1. A chlorophyll meter in use.

(model SPAD 502) enables users to quickly and easily measure leaf greenness which is affected by leaf chlorophyll content. Chlorophyll content or leaf greenness is affected by a number of factors, one being N status of the plant. Since the chlorophyll meter has the potential to detect N deficiencies, it also shows promise as a tool for improving N management.

The chlorophyll meter (see Figures 1 and 2) has several advantages over other tissue testing methods. A reading that indicates adequate nitrogen (or critical value) is not affected by luxury consumption; a plant will only produce as much chlorophyll as it needs regardless of how much N is in the plant. It is not necessary to send samples to a laboratory for analysis, saving time and money. Producers can sample as often as they choose, and can easily repeat the procedure if they question the results. Using a chlorophyll meter to monitor leaf greenness

¹Mention of a brand name does not imply endorsement by the University of Nebraska or the USDA Agricultural Research Service.

throughout the growing season can signal the approach of a potential N deficiency early enough to correct it without reducing yields.

Monitoring crop N status during the growing season accomplishes little unless it is possible to correct an N deficiency before it reduces yields. Using a chlorophyll meter as an N management tool is especially appropriate where additional N can be applied through the irrigation system (fertigation). Fertigation is generally limited to moving sprinkler systems due to the uniformity of application possible with center pivots or lateral move systems, although much progress is reported in the development of surge-flow fertigation systems. Fertigation applications in Nebraska must conform to state chemigation regulations which require certification of the operator and inspection of the required safety equipment (see your local Natural Resources District).

In non-irrigated cropping systems additional N fertilizer can be injected with a sidedress application if crops are not too tall, or broadcast or banded between rows using high-clearance equipment. One benefit of fertigation is that the applied N is rapidly taken up by the crop to correct the N deficiency. In a non-irrigated system, N application to dry topsoil may not be utilized until the next rain occurs, which may be too late for the plants to fully recover and produce optimal grain yields. Although the technique is quite new, we feel chlorophyll meters can be utilized on a large number of acres in Nebraska, and promises to improve fertilizer N efficiency and decrease risks associated with reduced fertilizer applications.

Many factors affect chlorophyll meter readings. Variety or hybrid differences can greatly affect the meter reading as some corn and sorghum hybrids are darker green than others. Stage of growth can affect leaf greenness as can recent environmental conditions such as temperature, moisture stress and sunlight. Plant diseases, nutrient deficiencies and nearly any other kind of plant stress can affect the ability of the plant to produce chlorophyll, thus affecting leaf greenness. Because the chlorophyll meter is affected by so many things, we are not able to say that a given meter reading indicates sufficient N. Meter readings mean very little by themselves and must be calibrated for each field, soil, hybrid and environment in order to make use of the readings. The best way to calibrate the meter is to maintain several adequately fertilized reference strips in each field.

Field Use of the Chlorophyll Meter

1. Establishing Reference Strips. For reasons outlined above, it is crucial that the chlorophyll meter be calibrated for each field, previous crop, hybrid, fertilizer and/or manure application and differing soil types. Several adequately fertilized reference strips, identical to the rest of the field except that they receive sufficient levels of nitrogen fertilizer, should be established in each field. The amount of N applied to these strips should be adequate to insure that plants in the reference strip do not exhibit an N deficiency. We suggest that the entire field be fertilized with one-half to two-thirds of the total amount of fertilizer N recommended by standard soil test procedures. The reference strips should then be established by applying additional N fertilizer so the total amount applied to

the strips is equal to or slightly higher than the N rate suggested by the University of Nebraska (see NebGuide G74-714, *Fertilizer Recommendations for Corn*). A large excess of N applied to the reference strips is not recommended and may reduce yields. Three to five reference strips should be established in each field to accurately represent conditions in that field (see *Figure 3*). By comparing the average chlorophyll meter readings from the reference strips to those from the rest of the field, N sufficiency and the need for additional N supplied through fertigation can be determined.

2. How to sample. Weekly chlorophyll meter readings from the reference strip and the bulk field should be compared at a minimum of three locations in each field. At each location, the average reading of 30 plants from the reference area and the adjacent bulk field should be compared. The Minolta SPAD 502 collects and stores up to 30 individual readings and calculates the average automatically. Care must be taken during collection of these readings to insure their accuracy. Individual readings will vary up to 15 percent from plant to plant, but the goal is to collect 30 readings so that the average accurately represents leaf greenness for that crop. Avoid taking readings from plants that do not represent typical plant spacings (e.g., wide guess rows, doubles or planter skips). It may be helpful to systematically sample each row across the planter width to avoid problems caused by differences among rows such as plant population, compaction or variations in starter or other fertilizer application.

The same leaf should be sampled from each plant. Avoid sampling very old or very young leaves. For plants between the six leaf stage (about 1 foot tall) and tassel, we recommend sampling the newest fully expanded leaf that has a leaf collar exposed (see *Figure 4*). After the tassel stage, sampling the ear leaf (the leaf attached to the primary ear shank) should give an accurate comparison. Readings taken from plants less than 1 foot tall are usually quite variable and probably not worth



Figure 2. Close-up of chlorophyll meter.

collecting. After selecting the leaf to be sampled, it is important to take the reading on about the same location on each leaf. We have found it works well to collect the reading from a point one-half the distance from the leaf tip to the collar, and halfway between the leaf margin or edge and the leaf midrib (Figure 4). Chlorophyll meter readings may vary with time of day, but as long as readings are collected from the reference strip and the bulk field at about the same time, the comparison is valid. It is best to avoid collecting readings whenever there is moisture on the leaves (i.e., after a rain or sprinkler irrigation or in the early morning) as this can distort the readings. Extreme temperature changes can cause meter readings to fluctuate, so the meter should not be left in the sun on a vehicle dashboard or taken from an air conditioned vehicle directly to the field on a hot day.

3. Interpretation of chlorophyll meter readings. After recording average meter readings from the bulk field and reference area at several locations in each field, an N sufficiency index can be calculated as follows:

$$\text{Sufficiency Index} = \frac{\text{Average Bulk Reading}}{\text{Average Reference Strip Reading}} \times 100\%$$

For example 1, we sampled four places in the field and obtained the following N sufficiency index values: 101, 98, 97, and 96 percent (see worksheet). Our experience shows that a sufficiency index lower than 95 percent indicates an N deficiency that should be corrected or it may lead to a yield reduction. In example 2 (see worksheet) the readings average less than 95 percent, indicating the need for additional N. At least 20 lbs N/acre should be applied through the irrigation system to correct the N deficiency, particularly since we know the crop is rapidly taking up N during this period. This field should be checked again with the meter 4-6 days after the N application to make sure the deficiency has been corrected or that recovery is under way.

Since the readings vary depending on weather and growing conditions, readings collected from a single sampling date are not as useful as comparing trends in the sufficiency index through the growing season. Readings should be collected weekly from the six leaf stage until about 20 days after silking. Fertilizations applied later than this do not increase yield.

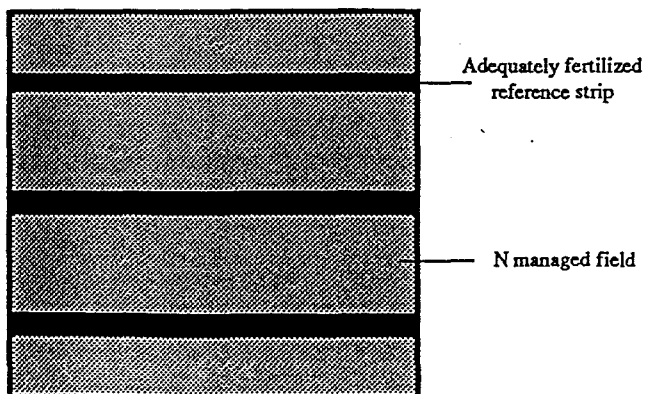


Figure 3. Field planted with reference strips.

4. The chlorophyll meter as an N management tool. It is important to realize that the chlorophyll meter enhances a producer's ability to make N management decisions but does not replace other aspects of good N management. Environmentally and economically sound N management must begin with a representative soil sample and a realistic value for expected yield (see also NebGuide G87-829, *Fertilizer Nitrogen Best Management Practices*). We suggest that at least one-half to two-thirds of the total fertilizer N be applied to the entire field prior to the six leaf stage in order to insure the chlorophyll meter technique is effective. If a corn plant experiences severe N stress in the early growth stages, the size of the ear and number of kernels can be limited so additional N fertilizer applied later will not allow full recovery of grain yield.

Using the chlorophyll meter to schedule fertigations allows adjustments throughout the season based on the amount of N supplied by mineralization of organic matter and manure, by contaminated irrigation water, or when plant roots come in contact with additional N.

When the need for additional N is indicated by the chlorophyll meter (e.g., N sufficiency index is at or below 95 percent, or a trend indicates it soon will be), an additional 20 to 40 pounds N per acre should be applied through fertigation. The decision on how and when to fertigate is affected by many factors including stage of growth, developing trends in chlorophyll meter readings, equipment limitations and anticipated crop N needs for the rest of the growing season. Most N fertilizer should be applied before the tassel stage. N applied more than 20 days after silking probably will not affect grain yields. Generally chlorophyll meter readings will respond to show crop recovery within two to three days after fertigation depending on environmental conditions.

The chlorophyll meter technique allows "fine-tuning" N management to field conditions and reduces the risk of yield-limiting N deficiencies. Producers should recognize this as another tool that may complement, but does not replace, other aspects of sound N management. One soil scientist said it succinctly, "Use the chlorophyll meter to schedule your last 50 lbs N/acre, not your first." Potential uses of these techniques in the future may include remote sensing by satellite or airplane to schedule the need for additional fertilizer N.

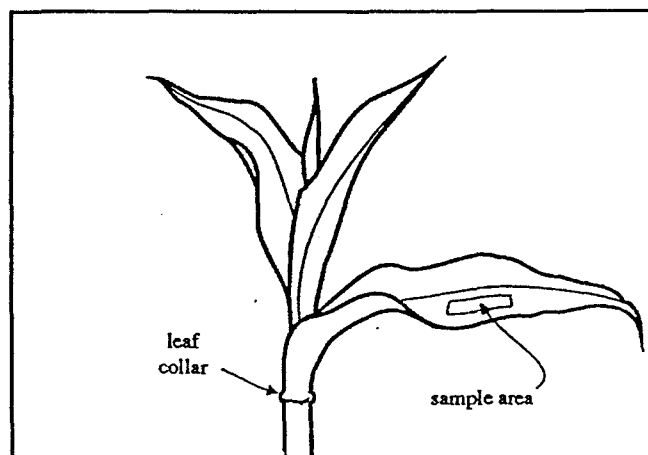


Figure 4. Sample area for taking chlorophyll readings.

Worksheet Example 1.			
Field: <u>North 80</u>		Date: <u>June 28</u>	
	Average Bulk Field Reading	Average Reference Strip Reading	N Sufficiency Index
Location 1	54.5	54.1	100.7%
Location 2	56.8	58.0	97.9%
Location 3	49.6	51.3	96.7%
Location 4	57.2	59.5	96.1%
Average			97.9%
Action: none required			

Worksheet Example 2.			
Field: <u>South 40</u>		Date: <u>July 10</u>	
	Average Bulk Field Reading	Average Reference Strip Reading	N Sufficiency Index
Location 1	50.2	52.4	95.8%
Location 2	48.2	51.6	93.4%
Location 3	51.3	54.4	94.3%
Location 4	54.0	58.1	92.9%
Average			94.1%
Action: Fertigate			

Your Data			
Field: _____		Date: _____	
	Average Bulk Field Reading	Average Reference Strip Reading	N Sufficiency Index
Location 1	_____	_____	_____
Location 2	_____	_____	_____
Location 3	_____	_____	_____
Location 4	_____	_____	_____
Location 5	_____	_____	_____
Average			_____
Action: _____			

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