Prediction of late nitrogen fertiliser effects on wheat quality using a chlorophyllometer

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Abstract

Wheat crops managed for high yield have the potential for increased grain protein content with applications of nitrogen (N) fertiliser at anthesis. The extent of the grain quality improvement is variable and dependent on environmental factors and crop management before and after application. The aims of the study were to determine whether increased protein quality could be predicted by rapid measurement of chlorophyll in leaves using a hand held chlorophyllometer, and whether improved yield and quality responses to the application of N fertiliser could have been predicted at a stage before or at anthesis. Nitrogen applied at anthesis did not influence grain yield but significantly increased grain protein content, as did application at sowing. Variation in flag leaf N concentration at anthesis was not strongly related to grain quality differences at maturity. Therefore, it was unlikely that chlorophyll measurements or leaf greenness at anthesis could be used to predict a potential grain protein response to anthesis N application. The chlorophyllometer was unsatisfactory for predicting the level of N in wheat leaves, primarily because of saturation of response at high levels of leaf N. However, there was potential for the use of a chlorophyll-based test for plant N diagnosis as leaf N was related to extractable chlorophyll in a strong linear manner.

Additional key words: *grain protein, crop monitoring*

Introduction

Wheat crops managed optimally for grain yield often have the potential for further increases in grain nitrogen (N) concentrations with timely application of nitrogen applied either as a foliar spray or solid fertiliser (Cooper and Blakeney, 1990; Gooding and Davies, 1992). An improvement in quality with N application at or around anthesis is dependent on the crop's capacity to take up N derived from soil N pools or fertiliser. A reliable field method to predict the potential quality gain is required to 1) reduce unnecessary fertiliser N inputs; and 2) quantify the amount of N fertiliser required to give a maximum increase in grain N concentration.

Fertiliser N application at anthesis has become a well accepted practice in wheat production. However, there are few methods available for use at the time of application for determining the potential economic benefits of this practice. This is especially important under high fertility conditions, as while a late N application may cause a protein increase, growers do not receive incentive payments for grain protein content in excess of 13%. Adequate soil moisture for plant N uptake is the primary consideration for decisions on whether or not to apply fertiliser, as this will generally ensure a yield response. The crop N status is also important since crops managed at a high level of soil fertility may not be responsive to the late application.

The principle benefit of using a chlorophyll meter over other tests is that results are available instantaneously. However caution is required in the interpretation of the values obtained from leaf samples because of possible instability in relationships between leaf chlorophyll concentration and leaf N content. The instrument produces an instantaneous, non-destructive estimate of the chlorophyll concentration (Marquard and Tipton, 1987) of the leaves. The method must be calibrated against quantitative N analysis. This requires destructive sampling followed by chemical and spectrophotometric analysis. Other rapid techniques for crop N monitoring include the semi-quantitative tissue tests (Scharf *et al.*, 1993) for measuring the unassimilated soluble contents of plant sap such as the Merckoquant NO_3^- test strips for wheat (Papastylianou, 1989) and Merckoquant test strips used with the portable 'Nitra-chek' apparatus for potato (Nitch and Varis, 1991). Critical nitrate levels can vary from site to site and can change rapidly over time, and therefore may not be a good indicator of the N status of the crops.

Chlorophyll meters have been used with some success for predicting N requirements of rice (Turner and Jund, 1991; Takebe *et al.*, 1990) and maize (Piekielek and Fox,

1992; Wood *et al.*, 1992; Wood *et al.*, 1993). However, there have been few reports of successful use of chlorophyll meters for prediction of N requirements of cereals (Follett *et al.*, 1992; Peltonen *et al.*, 1995). Good relationships were found for leaf N concentration and chlorophyll meter reading at late stages of development (anthesis). Predictions were carried through to grain yield but not for grain N concentration. There is some published evidence that suitability for use of leaf chlorophyll concentration to indicate potential response to fertiliser N may be dependent on the amount of non-chlorophyll N (Wood *et al.*, 1993).

In this project we attempted to determine whether rapid determination of leaf chlorophyll with a hand-held chlorophyllometer was an adequate surrogate for alternative measures of crop N status such as leaf N concentration or extractable chlorophyll. The aim of the field trials was to obtain measures of leaf greenness and chlorophyll concentration in plant tissue under variable fertility conditions, and to determine whether the responses could be related to improved yield and quality, and whether a beneficial application of N fertiliser could have been predicted at a stage before or at flowering.

Materials and Methods

Trial 1

A wheat (cv. Otane) trial was sown at Lincoln on 17 October 1995 at a sowing rate to achieve 300 plants per m^2. Plant establishment exceeded 80%. The trial was a randomised complete block design with 12 N fertiliser treatments replicated 3 times. Treatment combinations (Table 1) were arranged in a factorial design comprising 6 levels of N application during growth and 2 levels of N at anthesis. Nitrogen was applied by hand in the form of granular urea (46% N). Early N was applied on 3 November, followed by applications on 24 November (tillering), and late N was applied on 22 December (anthesis). Each plot was 1.4 m x 13 m (9 rows at 15 cm spacing).

A MAF quick test (12 October sampling date) at sowing showed generally good fertility (pH, 6.2; Ca, 12; K, 13; P, 24; Mg, 17; Na, 5; S, 5; organic carbon, 1.9%; total N, 0.19%; NH_4^+, 1 ppm; NO_3^-, 12 ppm). An independent soil nitrate test by aqueous extraction showed levels equivalent to 6.7 (±2.0) kgN/ha in the 0-30 cm depth.

A spray programme was designed to minimise fungal disease effects on leaf health. Folicure was applied at 2-week intervals from 20 November. At the first application folicure was combined with glean for weed control. Black Nightshade was controlled with 2 L/ha of MCPA on 30 November. The 1995/96 season was particularly dry during the grain filling period necessitating additional irrigations of 20 mm by overhead sprinkler. These applications were on 16 November, 6 December, 20 December, 3 January, and a final application of 25 mm on 11 January.

Chlorophyllometer measurement

Ten main stems were sampled from representative plants out of 0.1 m^2 quadrats harvested on five weekly occasions beginning 13 December. Stems were partitioned into flag leaves, flag-1 leaves, other green leaves, dead leaves, ears and remaining stem, dried at 80°C, and ground to pass a 1 mm sieve. Chlorophyllometer (Hardacre *et al.*, 1984) measurements were made on the flag and flag-1 leaves only, with duplicate readings taken at two positions on each leaf (base and middle). A 1-cm diameter leaf disk was punched from three positions (base, middle, and a position mid way between the two) for chlorophyll extraction. The remaining 10-leaf portions were combined, dried and ground. Samples were analysed for N and carbon content by the Dumas total combustion method (Fox *et al.*, 1994) using a LECO CNS-2000 elemental analyser.

Chlorophyll extraction

The 10 disks from each position for each leaf type were stored in plastic envelopes and frozen until subsequent chlorophyll extraction. Chlorophyll content

Table 1. Treatment combinations for Trial 1, Lincoln.

	N application (kg N/ha)			
	Early-season			
Treatment	Sowing	Tillering (GS3)	Anthesis	Total
1	0	0	0	0
2	0	0	50	50
3	25	0	0	25
4	25	0	50	75
5	25	25	0	50
6	25	25	50	100
7	50	0	0	50
8	50	0	50	100
9	50	50	0	100
10	50	50	50	150
11	100	100	0	200
12	100	100	50	250

of bulked leaf disks was determined by extraction in darkness by agitating for three hours in 4 ml of N,N dimethlyformamide, followed by colorimetric determination on a Shimadzu UV-160-Spectrophotometer at 664.5 and 647 nm. The chlorophyll concentration was calculated according to an equation (Marquardt and Tipton, 1987), and adjusted to chlorophyll content (ng/cm^2) per unit leaf area.

Dry matter harvest

At the anthesis harvest no quantitative information was obtained for determining the level of N uptake. At maturity (16 February), a single 0.2 m^2 quadrat sample was taken from each plot for N uptake and residual N determination in plant tissues. In addition, a 10-plant subsample was partitioned into grain, chaff, leaf and stem fractions. Samples were dried and ground for LECO analysis for N concentration. Mean grain size was determined as 1000 kernel weight (g). Grain protein was determined from %N x 5.7.

A single one m^2 quadrat sample was taken from each plot on 22 February for the best estimate of grain yield. Moisture content was measured using a Foss moisture meter and grain weight corrected to 14% moisture content. An additional yield sample was taken on 22 February using a Wintersteiger plot harvester and yield corrected to 14% moisture. Grain samples were ground prior to N analysis. This sample was used as the most representative measure of grain quality.

Trial 2

A wheat trial (cv. Domino) was conducted at Methven on a Paparua sandy loam with treatment combinations as shown in Table 2. The design was a randomised complete block with 2 replications. Treatments 1, 2 and 3 only were part of the designed experiment. A low-N treatment (4) was included for comparison with the former treatments managed for high yield. The crop was sown in mid May. Quadrats (0.2 m^2) were sampled on three occasions at weekly intervals beginning at anthesis (4 December). Chlorophyll absorbance was measured on a representative sample of 10 flag leaves at a position adjacent to the leaf ligule and at a position mid way along the leaf length. Flag leaves were bulked for N analysis by the LECO method as for the Lincoln trial.

Table 2. Treatment combinations for Trial 2.

	N application (kg N/ha)		
	Pre-anthesis		
Treatment	GS2	GS5	Anthesis
1	80	80	0
2	80	80	40
3	80	80	80
4	40	0	0

Quadrat samples (0.2 m^2) at the successive samplings at anthesis and early grain filling were used for calculations of dry matter accumulation, N content, and N uptake for each of the component plant fractions (dead leaves, green leaves, stem and ears). At maturity, plants were partitioned into dead leaf, stem, grain and chaff samples. In addition, a 1.2 m^2 quadrat was taken for grain yield, 1000 kernel weight, percent screenings (above and below a standard 5.5 sieve), and grain N concentration.

Results and Discussion

Trial 1

Chlorophyllometer calibration

The chlorophyllometer potentiometer settings gave meter readings spanning the range -5 to +20 on the low sensitivity selection. Green leaves were typically in the range of 12-17, while senescing leaves with pale green or yellow colour were in the 0 to 12 range. In the green band, the meter reading was near-linear when plotted against extractable chlorophyll (data not shown). However when plotted over the whole range of extractable chlorophyll, the relationship was non linear. This relationship was near-linearised by plotting the log-transformed chlorophyll content against meter reading (r^2=0.72). Deviations from the linear response may be due to significant 'non chlorophyll' N (amides, amines and non protein-bound chlorophyll), (Wood *et al.*, 1993). Prior calibration of the meter against a range of optical density filters showed a tight curvilinear response (data not shown). This indicated a direct relationship between meter response and the level of chlorophyll absorbance. However, there was a tight clustering of observations at the upper end of the response range.

Chlorophyll meter response

Pre-anthesis and anthesis sampling dates were used to establish standard procedures for chlorophyll meter measurements with regard to sampling of leaf number and position along the leaf.

Chlorophyll meter readings were significantly different for flag leaves compared with the subtending (flag-1) leaf ($P<0.001$). The meter was therefore able to discriminate leaf greenness levels of neighbouring leaves within a particular N fertiliser treatment at anthesis.

Overall N fertiliser effects were also significant ($P<0.01$) and the interaction between fertiliser level and leaf position was significant ($P<0.05$). Therefore, the relative difference between flag and flag-1 leaves varied with the fertiliser level. The differences between leaves were greater at the lower fertiliser rates.

Chlorophyll meter readings were also taken at three positions along the leaf. The main effect of leaf position was highly significant ($P<0.001$), and there was a strong interaction with N fertiliser treatment. Multiple measurements (among 10 plants) within leaf position were not significant and this was not affected by N treatment. These results showed that definition of a reliable sampling method was possible in the field situation with fewer than 10 observations per plot. Most importantly, there was significant discrimination between the N treatments and observations were sufficiently reliable to detect differences between leaves on the main stem culm and position along the leaf.

Selection of leaf sampling position is an important consideration both from the point of view of repeatability and also for optimising the differences between plants and leaves grown under differing fertility conditions. For subsequent analysis and purposes of simplicity, a 'middle' location along the leaf was considered the most appropriate. Flag leaves were also considered the most appropriate as they were invariably wider and more easily measured. More recently formed leaves are also more likely to be representative of the current N status of the plant.

Relationship between chlorophyll meter reading and leaf nitrogen

Leaf chlorophyll in most crops is directly related to leaf N (Lopez-Cantarero *et al.*, 1994). In our experiment, chlorophyll meter readings plotted against leaf N concentration showed that near-saturation occurred at leaf N concentration in excess of 3%, (Fig. 1A). While there was a slight linear trend above the 3% N level, the response over the entire leaf N range was curvilinear. Discrimination was therefore only possible between leaves of very low N concentration and those with adequate N concentration.

There was a significant improvement in terms of linearity and range for the relationship between extractable chlorophyll and leaf N % ($r^2=0.75$; Fig. 1B). This relationship shows there is a valid conceptual basis for use of a chlorophyll meter to detect differences in leaf N concentration.

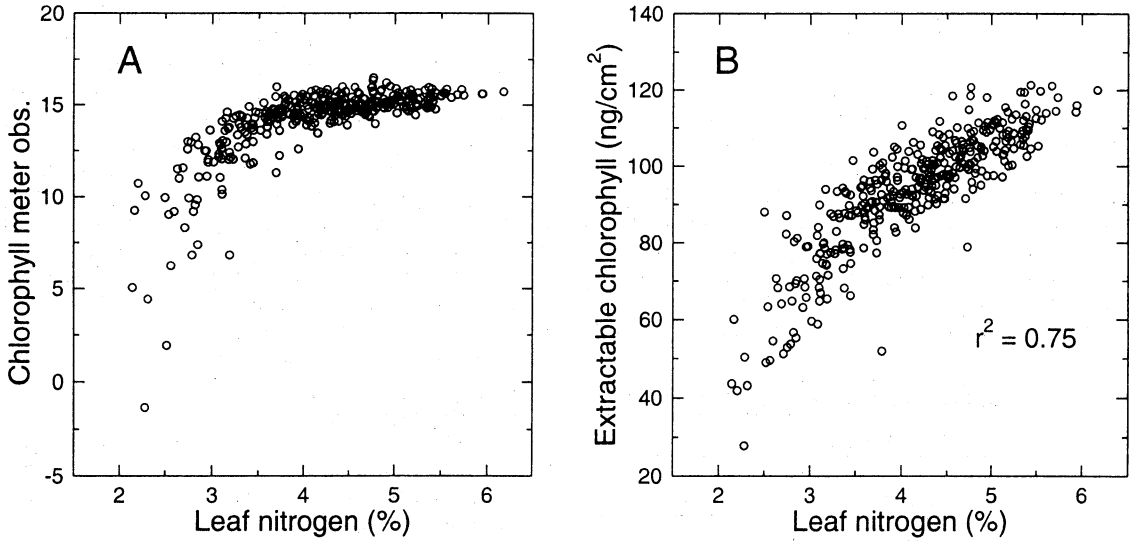

Figure 1. Relationship between A) chlorophyll meter reading and leaf nitrogen, and B) extractable chlorophyll and leaf nitrogen. Data were for individual plot means over five sample dates at Lincoln (Trial 1).

Yield responses to fertiliser N

Grain yield (1 m^2 quadrat) among all treatments ranged from 4.5 to 6.3 t/ha with a curvilinear relationship (r^2=0.49; P=0.02) in response to the total amount of N applied (Fig. 2A). The pre-anthesis N treatment effect was significant (P < 0.001) for both harvester yield and quadrat yield. The effect of anthesis N was not significant, but the interaction between timing and rate was marginally significant. A yield improvement due to anthesis N application occurred at the higher levels of pre-anthesis application, (treatments 8, 10, 12). In these treatments, increased grain numbers (through increased tillering) increased the potential yield response to late N. The effect of late N application on kernel weight was not significant. Therefore, the small yield enhancement with anthesis N was most likely due to higher grain set.

Grain quality responses to fertiliser N

The effect of anthesis N on grain protein content was strongly significant (P<0.001) for both harvester samples and quadrat samples (Fig. 2B). Likewise, the grain protein improvements were strongly significant over the range of pre-anthesis application levels and there was a significant interaction between pre-anthesis N application and timing (± N at anthesis). Crops which were adequately fertilised had a reduced capacity to respond to the late N application. The upper limit of quality was achieved with between 150 and 200 kg N/ha. Protein content was improved by 2.2% in treatment 2 (14.4%) compared to the control (no early N). Protein content exceeded 15% at the high early-season N application rates and there was no response to late N. The mean improvement in protein content due to late N application was 0.8% for both quadrat and combine harvested plots.

On the basis of these yield and protein responses to anthesis N application, crops at the low end of the yield response curve will be best served by diagnostic methods which help determine whether the late N application is beneficial. All crops in this trial were grown under adequate soil fertility conditions, and this was reflected in the comparatively small yield difference between the low and high fertility treatments. Indeed, there were no apparent visual differences between treatments in general leaf colour, although small differences were noted in the stand density between low and high fertility treatments. Nitrogen uptake per unit area determined at maturity was not significant for fertility treatment level nor anthesis application timing for any of the plant fractions (dead

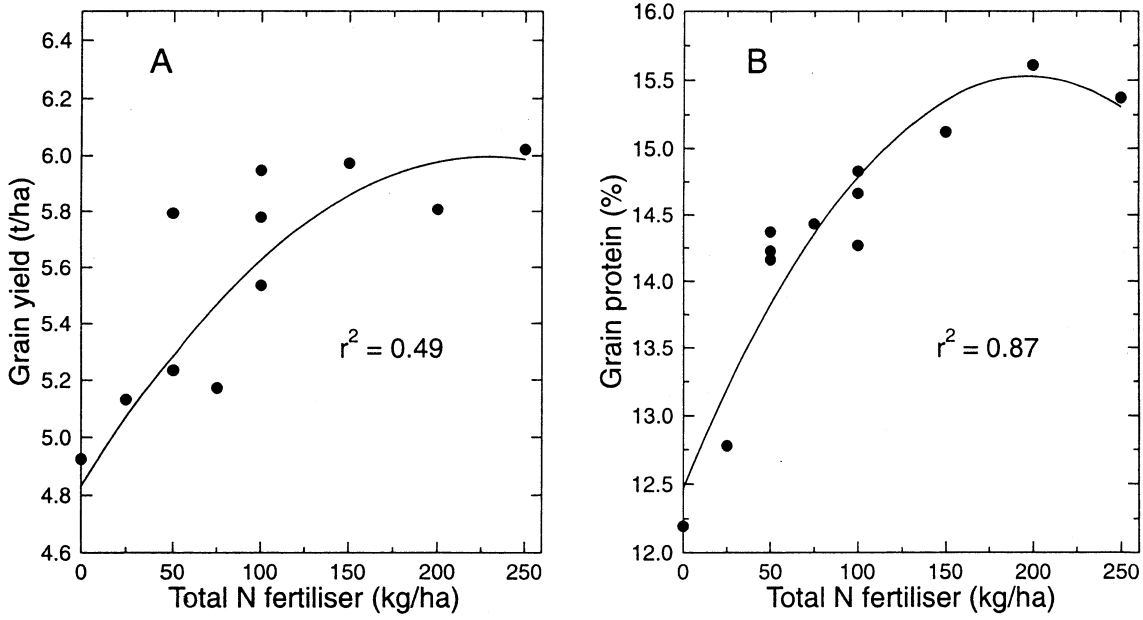

Figure 2. Response of A) grain yield to total nitrogen application (pre-anthesis + post-anthesis applications) for treatment means and B) grain protein to total fertiliser application. Solid lines show the quadratic fits.

leaf, stem, grain or chaff). The effects for the whole plant were also not significant.

In this trial, there were strong indications that the crops were generally in a state of positive N balance, irrespective of the N fertiliser treatments. At maturity, there was significantly ($P<0.001$) greater residual N in the dead leaves and stems in crops with anthesis N application compared to crops without. The effect of pre-anthesis N level was also strong ($P<0.001$). The interaction was not significant. In all treatments, the 50 kg of N applied at anthesis was therefore not fully utilised for translocation to the developing grains.

Predictors of yield and quality at maturity

Leaf N concentration, extractable chlorophyll and leaf greenness (flag leaf chlorophyllometer readings) were related to final grain yield for each of five successive weekly samplings. In all cases, the relationships were positive but depending on the sample timing were not universally significant. Best relationships of yield to flag leaf N content occurred one week before ($r^2=0.39$) and one week after anthesis ($r^2=0.38$). Data for the anthesis sample are given in Fig. 3A. Similarly, flag leaf chlorophyll content was most strongly related to grain yield one week before anthesis ($r^2=0.26$), but was not significant at the anthesis stage (Fig. 3B). The flag leaf chlorophyllometer reading at anthesis was strongly related to the grain yield, ($r^2=0.56$, Fig. 3C), a result which is notable given the time delay inherent in the prediction. In all cases, the yield range was comparatively small (4.3-6.6 t/ha) and consequently the predictive potential of these relationships is reduced.

Positive relationships between grain quality and flag leaf N occurred over the sampling period (1 week before through to 3 weeks after anthesis) with the strongest relationship occurring at the earliest sampling ($r^2=0.47$) and anthesis (Fig. 4A). Again, the predictive capacity was limited by the limited range of both grain quality and leaf N variables. There was little or no relationship between extractable chlorophyll and grain quality (Fig. 4B). Similarly, leaf greenness was not related to grain quality except at the anthesis harvest ($r^2=0.39$; Fig. 4C). This result is possibly a fortuitous one, certainly one that could not be reliably used in the field.

The predictive capacity of the chlorophyll meter technique is limited primarily by the saturation of the response range for leaves in treatments where there may be an expected gain in the quality of grain. This is especially so in the high fertiliser N rates. If data in the upper response range were omitted there would be an improvement in the relationship. Also, the range in chlorophyll meter readings could well be improved with

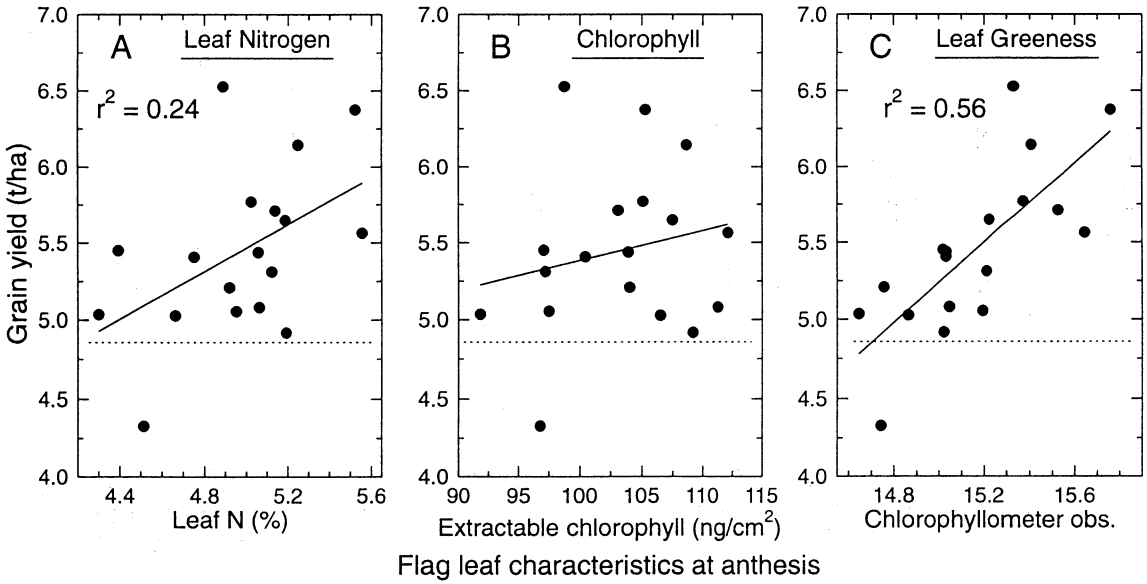

Figure 3. Relationships between grain yield and A) leaf nitrogen B) extractable chlorophyll, and C) chlorophyll meter reading. Data from the Lincoln trial, individual plot means.

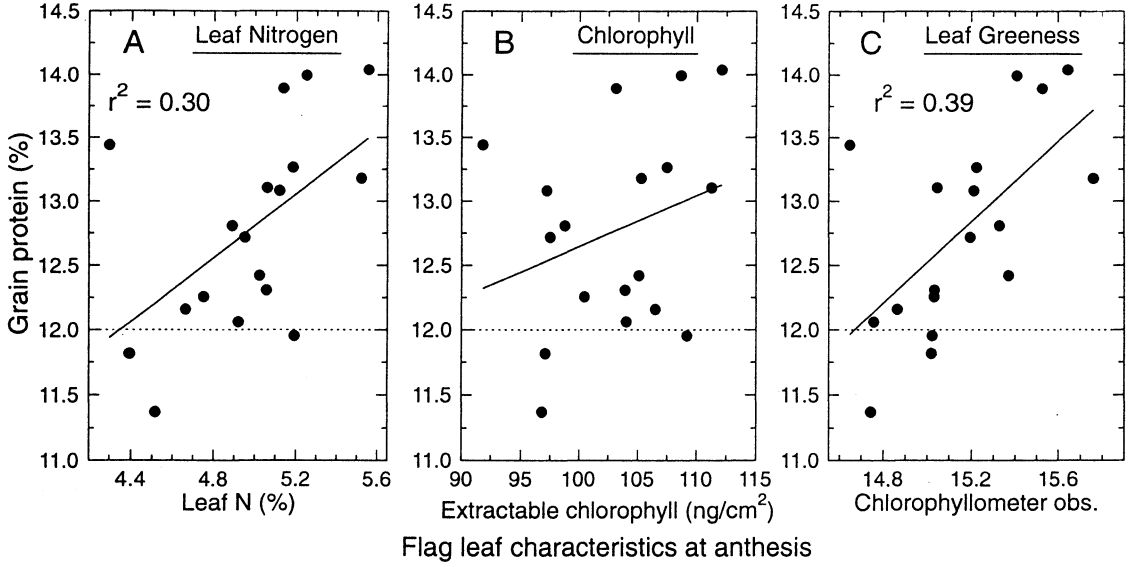

Figure 4. Relationships between grain quality (protein %) and A) leaf nitrogen B) extractable chlorophyll, and C) chlorophyll meter reading. Data from the Lincoln trial, individual plot means.

modification to the hardware so that it was more sensitive in the upper range of chlorophyll concentrations. The thickness of the wheat leaves may also exacerbate the problem of achieving reliable meter readings. Further evaluation of leaf measurement lower in the culm would not be a good prospect, especially in N-deficient crops or crops sown at high densities because only two or perhaps three leaves were undamaged or had not begun to senesce by the time anthesis occurred. Young leaves contain less chlorophyll than older leaves, and it is conceivable that the chlorophyll meter could be used to track the rate of development of chlorophyll which in turn may be related to the N economy of the plant.

Prediction of yield and quality enhancement due to late N fertiliser application

None of the predictor variables (flag leaf N concentration, flag leaf extractable chlorophyll, chlorophyll meter reading) measured at anthesis were identified as useful predictors for yield enhancement due to anthesis N fertiliser application. Mean yield enhancements over all pre-anthesis N treatments were less than 1 t/ha and approximately half of the plots showed a decline in yield with fertiliser addition at anthesis.

The mean protein content increase due to anthesis N application from quadrat samples was 0.7%. It was questionable whether this increase was predictable at anthesis. There was a trend for reduced magnitude of quality enhancement as the value for the predictor variable increased (Fig. 5A, B and C). In all cases, the slopes of the responses were not significantly different from 0. In all but two instances increasing levels of leaf N, flag leaf extractable chlorophyll, and flag leaf chlorophyllometer readings at anthesis resulted in increased grain quality but reduced response to late N.

Trial 2

Yield components and nitrogen content

At maturity, there were few significant effects due to level of N application at anthesis. There was, however, a significant effect on total plant N uptake and N accumulation in the grain (Table 3). Grain protein concentration did show a trend toward higher levels with increased rates of N applied at anthesis (13.5% for the

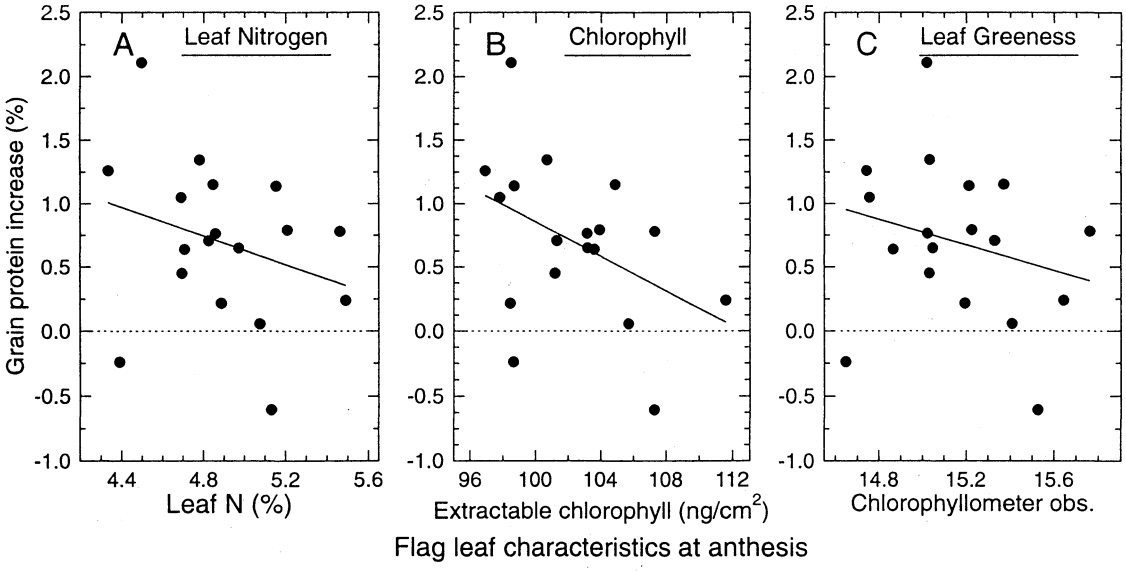

Figure 5. Relationships between percentage grain protein increase due to N applied at anthesis and flag leaf characteristics at anthesis. Predictors used in plot A) leaf N, B) extractable chlorophyll, and C) chlorophyll meter reading. Data from the Lincoln trial, individual plot means.

control vs 15.5% for 80 kgN/ha treatment). Similarly, there was a non significant trend toward increased protein in screenings (<5.5 standard screen). Protein content was significantly improved for grain not passing the screen (>5.5 screen, Table 3). Protein content in the low N input treatment (4) was substantially lower than in the high N management trial area, but this could not be tested statistically. There was also no effect of late N level on mean grain size (TKW) or grain yield.

Leaf nitrogen

An attempt was made to relate the apparent 2% gain in grain N content due to anthesis N application to the flag leaf N content and/or the associated chlorophyllometer measurements made at anthesis and two dates thereafter. Mean flag leaf N contents at anthesis in the 'high N management area' were 4.4% (±0.3) compared to the 'low nitrogen' area of 3.1% (±0.05). These levels did not change appreciably in the

Table 3. The effect of anthesis N on grain yield, grain protein, 1000 kernel weight (TKW) and N uptake at Methven (Trial 2).

Treatment (anthesis N level)	Yield (t/ha)		Protein (%)			TKW	N uptake (g N/m²)	
	Grain (14%)	Biomass	Whole sample	Screening >5.5	Screening <5.5	(g)	Grain	Whole plant
1. (control)	6.36	20.9	13.5	11.7	16.8	35.3	18.3	25.2
2. (40 kg N)	6.20	20.3	14.9	14.5	15.0	35.8	22.1	31.0
3. (80 kg N)	6.56	23.2	15.5	14.7	17.0	37.3	32.4	40.8
4. Low N (control)	4.92	17.6	12.8	10.2	14.5	35.2	15.3	22.4
Significance[1]	n.s.	n.s.	n.s.	**	n.s.	n.s.	*	*
$L.S.D_{(0.05)}$				1.6			6.4	12.6

[1] n.s. = not significant; * and ** indicate significance at P< 0.05 and 0.01 respectively.

following week, but there was a trend toward elevated N concentrations in the 80 kgN/ha treatment in the following week (4.77%).

Leaf nitrogen vs chlorophyllometer measurement

The mean (of 10) leaf base measurements by chlorophyllometer measurements were 0.73 (±0.34) units higher than mid-position measurements. These were highly correlated (r=0.93) irrespective of sampling time, indicating stable relative within-leaf consistency in chlorophyllometer measurements. Both measurement consistency and range are paramount for successful detection of leaf N variation. It was also important to determine whether differences in field chlorophyllometer measurements were reflected in differences in flag leaf N concentration. Indeed, there was no significant relationship between these variables within the high N management area (Fig. 6), and certainly no statistical effect due to level of N application at either of the subsequent application dates. The chlorophyllometer was therefore not able to detect an enhancement in leaf colour as a result of anthesis N application.

The relationship between chlorophyll meter observations and flag leaf N (Fig. 6) was significant only when the low N management data were included, but these observations had a strong influence on the overall relationship. These data included the anthesis and two post-anthesis sampling dates. Within the 'high N management' trial area there was no consistent discrimination with respect to leaf N concentration. The differences between the 'high N' and 'low N' management areas were detectable by eye as a distinct difference in leaf greenness. The chlorophyllometer was only able to detect differences in N concentrations at levels in the leaf below 4% N.

Practical implications

Grain quality enhancement was observed in both trials with anthesis application of fertiliser N and growers should continue this practice when there is an expected quality gain. A decision not to apply N would generally occur in crops with significant soil moisture deficit at the time of intended fertiliser application and with 'near maximum' level of crop N. In this case, there is little likelihood of improvement in grain N concentration. Nitrogen for grain will primarily be supplied by remobilisation from reserves and late uptake from the soil.

Premium prices for high protein were set at 13.1% protein for the cultivars Otane and Domino in the 1995/96 season. Protein content substantially in excess of these values is not recommended for milling purposes. Therefore, late N for yield enhancement in high fertility situations would give little economic benefit to growers. However, on less fertile land, late N will reliably enhance protein content and the chlorophyllometer may well be useful for determining whether the late N application is warranted. Further research is required to determine whether alternative soil and crop monitoring methods are useful for mid-season management decisions. Such methods may include regular soil mineral N measurement, soil moisture monitoring, and determination of crop potential for N uptake during grain filling. Rapid determination of nitrate in soil and plants, and ammonium in soils using test kits or specific ion electrodes are possible alternatives.

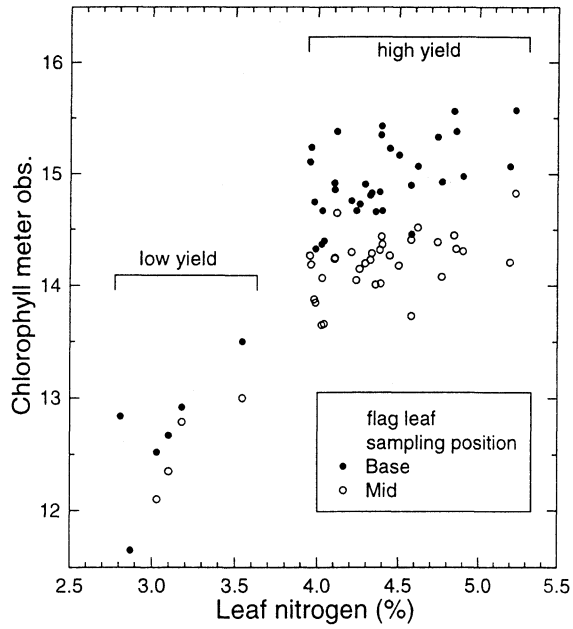

Figure 6. Relationship between chlorophyll meter observation and flag leaf nitrogen concentration for high yielding (treatments 1, 2, 3) and low yielding (treatment 4) plots at Methven. Data include base and mid-leaf position measurements for the anthesis and two following sample dates.

Conclusions

This study showed that the chlorophyllometer has limited practical use for predicting the potential response of grain yield and grain N to anthesis-applied N fertiliser. The chlorophyllometer may, however, be useful for predicting potential grain N increases at the lower end of the yield response curve, when responses of leaf characteristics to applied fertiliser are more pronounced. Crops in this study were managed for high yield and while the added benefit of further N application at anthesis was effective in raising the grain protein concentration by up to 2%, this positive effect was not detectable with the chlorophyllometer prior to grain filling. The difficulty of using a diagnostic test in these situations was more a consequence of the narrow range in plant tissue N and chlorophyll concentrations at flowering than the inability of the chlorophyllometer to detect the subtle differences in chemical components containing N. Leaf N was related in a strong linear manner with extractable chlorophyll. Therefore, possibilities remain for the use of a chlorophyll-based test for plant N diagnosis. However, under high N management regimes it is unlikely that chemical tests alone (including total N determination of the whole plant or individual plant parts, or leaf C:N ratios) on plant components at earlier stages of crop development will be successful in predicting end-of-season wheat quality or the effectiveness of late N application.

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