Comparison of field indicators of nitrogen status in perennial ryegrass (*Lolium perenne* L.)

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Abstract

Prediction of plant nitrogen (N) requirements is necessary for efficient N-use within the requirements for sustainable production systems. Herbage N concentration is related to yield (both for herbage and for seed) but requires laboratory analysis, which is time-consuming. A comparison of analyses indicating N status in perennial ryegrass using dry weight, herbage N concentration, in-field sap nitrate (colour measured 'by eye' and with a meter) and chlorophyll meter readings is presented. Sap nitrate, whether subjectively (by eye) or objectively (meter) recorded, was not a good indicator of herbage N concentration. The chlorophyll meter could predict herbage N status (R^2= 0.78) and readings were operator-independent, indicating that it has potential for use as a field tool in identifying nitrogen deficiency.

Additional key words: *chlorophyll meter, herbage N concentration, sap nitrate*

Introduction

In most environments, availability of nitrogen (N) is insufficient to meet plant requirements (Grindlay, 1997) unless fertiliser-N is added. In an attempt to match yield potential of new plant cultivars, increasing amounts of fertiliser-N are being used; in New Zealand, for instance, the amount of N-fertiliser applied has trebled since 1990 (Bolan and Podila, 1996). Crop yields, however, have not increased to the same extent. The problem is that N is rarely used efficiently (Peoples *et al.*, 1995) as nitrogen is cheap to purchase and growers tend to have an 'insurance' approach to its use.

Environmental concerns about pollution are resulting in limits being placed on N-use in some parts of the country (e.g., 150 kg/ha N *per annum* in Marlborough and Nelson and 200 kg/ha N *per annum* in Wellington (Painter *et al.*, 1997); in Canterbury a limit of 200 kg/ha N *per annum* is being discussed (Johnson, R., Canterbury Regional Council, pers. comm.)). Research emphasis in cropping is now on using inputs, such as nitrogen, efficiently (Rowarth, 1997) to produce optimal rather than maximal returns.

Results from N-fertiliser response field trials cannot be extrapolated from one site and year to another with confidence (Rowarth and Archie, 1994; Rowarth and Cornforth, 1997). As a consequence, attempts have been made to find indicators of plant-N status which can be used during the growing season to identify whether the crop is under stress (Buwalda, 1984). An ideal indicator would be:

1. related directly to yield
2. available sufficiently early in growth to be able to modify outcomes
3. reliable
4. rapid
5. simple and cheap

The most widespread approach to assessing plant-N status has been to measure combined-N, usually expressed as % N in the dry matter (Grindlay, 1997). Herbage N concentration has been shown to be related to yield of ryegrass both in its vegetative (Cornforth, 1984; Smith *et al.*, 1985) and reproductive (Rowarth and Archie, 1994, 1995) stages, and uses the plant as the integrator of all sources of N (fertiliser, soil and atmosphere (Rowarth and Archie, 1994)). However, although analysis of herbage N concentration meets the first three criteria, it cannot be performed in the field to give an instant indication of N status and likely response to fertiliser. Sap nitrate tests do meet the last two criteria but although they have been used with success in maize (Cornforth, 1980) and evaluations have indicated potential for use in other cereals (Withers, 1982; Withers and Palenski, 1984; Palenski-Brown and Kemp, 1989), no data are available for perennial ryegrass. Similarly, chlorophyll meters have been used to reveal N-deficiency with success in several crops (for a review see Wood *et al.*, 1993), but no data are available for perennial ryegrass.

This research was established to identify the relationship between herbage N concentration (as it has been shown to be related to yield), sap nitrate and chlorophyll (as indicated by a chlorophyll meter) in perennial ryegrass (*Lolium perenne* L.). The physiological relationship between the pools which these analyses are measuring is shown in Figure 1. Variability in readings according to operator was also evaluated.

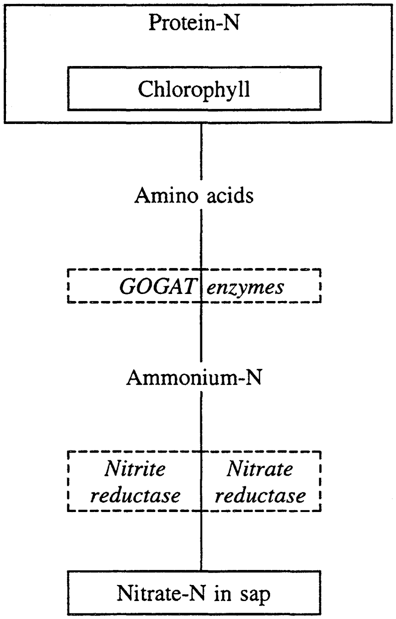

Figure 1. Schematic representation of the relationship between nitrate-N, herbage N% (protein-N) and chlorophyll in the plant.

Materials and Methods

Twenty trays of perennial ryegrass cv. Grasslands Nui were sown on 2 June 1997 in potting mix fully supplied with nutrients other than nitrogen. Five nitrogen treatments (0, 25, 50, 75 or 100 kg N/ha, applied as urea) were imposed at sowing. An additional 5 or 10 kg N/ha were applied to two trays out of the four for each treatment on 30 June to create a range of herbage nitrogen concentrations. The trays were located in the Hilgendorf glasshouse, Lincoln University with mean minimum and maximum temperatures of 15 and 25°C, respectively. All analyses were performed on 7 July, 1997.

Dry weight was measured from herbage cut at 'ground' level from a 10 x 10 cm quadrat, dried overnight at 80°C.

Herbage samples were analysed for herbage nitrogen (N%) (Basson, 1976) by the AgResearch Soil Fertility Service.

Sap nitrate readings were taken within two hours of dawn. Sap was expressed from the fleshy part of the stem using a garlic crusher and the sap wiped onto the nitrate pad of a Merckoquant nitrate strip from a freshly-opened container. The colour change was assessed subjectively after comparison with the scale on the Merckoquant nitrate strip packet. Any readings that reached maximum colour within the time allocated (60 s) were converted to sap nitrate (mg/L) using the following equation (derived from Cornforth, 1980):

$$y = 103082x^{-1.1513}$$

where y = nitrate (mg/L) and x = time (seconds). At the same time, on a duplicate sample and Merckoquant strip, the 'Nitracheck meter' was used to give an objective assessment of colour change.

A Minolta chlorophyll meter SPAD-502 is a small hand-held meter that provides non-destructive SPAD (unitless) readings, which are related to chlorophyll concentration (Wood *et al.*, 1993). An average of 10 readings was obtained for each tray of ryegrass. Readings were taken from the first most-fully expanded leaf, 25 mm from the ligule. This process was repeated three times by three individuals.

Data were analysed using Minitab statistical package (to perform ANOVA and linear regression analysis) and Excel graphics. Nitrogen uptake was calculated from dry weight x herbage N concentration.

Results and Discussion

Initial calculations were performed using the data from only the ten trays with base nitrogen treatments to avoid possibility of confounding dry weight measurements where late N had affected herbage N% but not growth. Increasing the amount of nitrogen applied to ryegrass resulted in a significant increase in herbage N% and chlorophyll (as indicated by SPAD) (Table 1). Overall relationships (as indicated by R^2) were significant for dry weight, herbage N, N uptake and SPAD.

Regression analyses (Table 2) indicated that herbage N% was significantly related to both dry weight (R^2 = 0.60) and SPAD (R^2 = 0.67). The relationship was improved (R^2=0.75), however, using both factors in a multiple regression, although the confidence in the accuracy of the prediction decreased slightly. Success with this approach has been achieved in wheat (Reeves *et al.*, 1993) where the multiple regression model was shown to account for 81% of the variation in yield.

The full data set was used to explore the relationships, excluding dry weight, more fully. Sap nitrate was related to herbage N% (Fig. 2), but the former could not be used to predict the latter: no sap nitrate could be measured until herbage N% was above a critical value of approximately 3.5% (using Cate and Nelson (1965) separation techniques), after which a very rapid increase in sap nitrate was measured which bore no relationship to increase in herbage N%. A similar threshold (3.2%) has been reported for sap nitrate accumulation by Smith *et al.* (1985). As they used a laboratory-based extraction for sap nitrate, their method is likely to have been more sensitive than the nitrate-strip field technique used in the current research.

Table 1. Effect of N-fertiliser on perennial ryegrass dry weight, herbage N, N uptake, sap nitrate and SPAD. Data presented are for initial N treatments only (n=2).

Treatment (N kg/ha)	Dry weight (g)	Herbage N (%)	N uptake (mg)	Sap nitrate (mg/litre)	SPAD
0	0.97	1.79	17.0	27	11.6
25	1.44	3.07	44.2	122	20.1
50	1.52	4.29	65.2	5554	22.0
75	1.54	4.99	76.8	3664	22.5
100	1.88	5.37	101.0	10658	22.4
$LSD_{0.05}$	ns	1.30	ns	ns	3.5
Regression					
R^2	0.49	0.87	0.75	0.03	0.62
Signif.[1]	*	***	***	ns	**

[1] level of significance * = 0.05, **=0.01, ***=0.001.

Table 2. Linear regression equations and statistical significance for prediction of herbage nitrogen. Data used in calculations were from initial N treatments only (n=2).

Regression equation	R^2	P
N% = -0.46 + 2.98 Dry weight	0.60	0.005
N% = 3.38 + 0.000129 sap nitrate	0.22	0.098
N% = -1.46 + 0.275 SPAD	0.67	0.002
N% = -1.90 + 1.59 DW + 0.178 SPAD	0.75	0.003

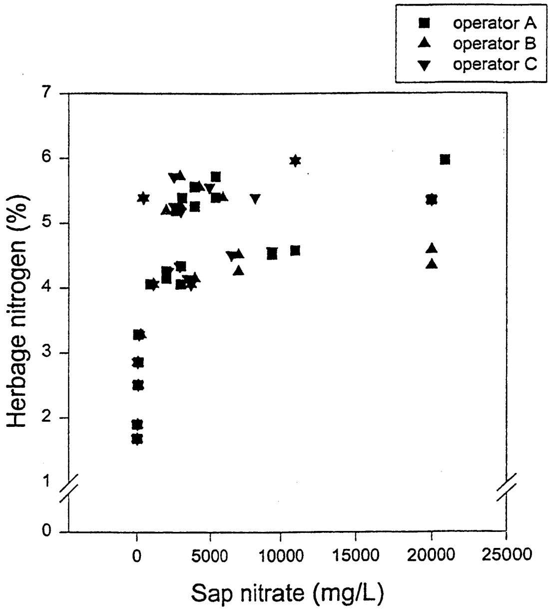

Figure 2. Relationship between sap nitrate (mg/L), assessed by eye using Merckoquant strips, and herbage N (%) in perennial ryegrass, as affected by operator.

Variability between operators using the nitrate-strips was considerable (average standard deviation for each tray = 36.2%) and inconsistent (i.e., no one operator always read higher or lower than the others). Using the Nitracheck meter to remove operator variability did not improve the relationship between herbage N% and sap nitrate (Fig. 3) as practical difficulties were encountered. Firstly, low-N herbage was very dry and extracting sap was difficult. This resulted in patchy application to the strips, which resulted in patchy colour development. The meter integrated the patches to give an overall reading, whereas the human eye was able to read only the coloured part. Secondly, the high-N herbage contained more sap nitrate than the meter could read; that is, colour development passed 500 mg/L in 60 seconds, but the machine could not be set to read in less than 60 seconds. The instructions suggested that dilution of the sap would

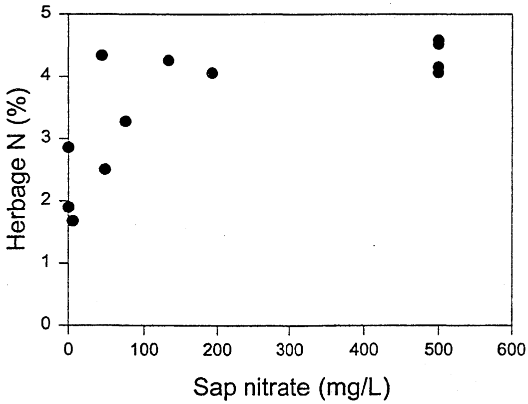

Figure 3. Relationship between sap nitrate (mg/L), assessed using Merckoquant strips and a meter, and herbage N (%) in perennial ryegrass.

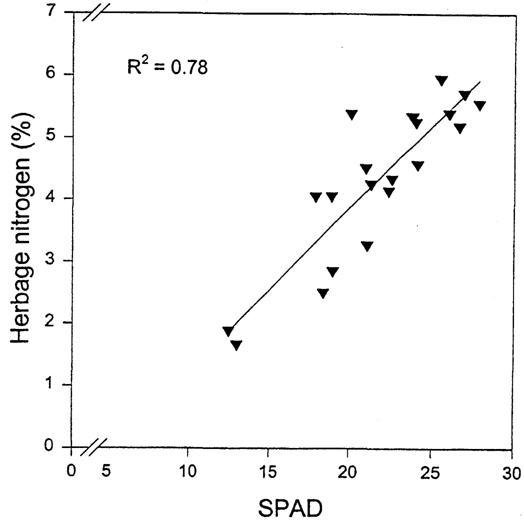

Figure 4. Relationship between SPAD and herbage N (%) for one operator.

be necessary under these circumstances, but with ryegrass, which does not have large, sappy stems, this is not practicable in the field.

A further problem associated with measuring sap nitrate is the restriction on time. Traditionally sap nitrate is measured within two hours of dawn as nitrate reductase, the first enzyme in the sequence of reduction steps from nitrate to ammonium (Fig. 1), is induced by light intensity and the presence of nitrate (Andrews *et al.*, 1992). It is also temperature sensitive and activity is reduced by the presence of ammonium. As a consequence, the size of the nitrate pool is highly changeable. The factors affecting nitrate reductase activity in ryegrass have yet to be fully elucidated.

The relationship between SPAD and herbage N% was positive and linear (Fig. 4). SPAD accounted for 78% of the variability in herbage N% indicating that SPAD could be used as an indicator for herbage N% in ryegrass, as it can in other crops (Wood *et al.*, 1993). Furthermore, incorporating the results from three operators (Fig. 5) reduced the reliability of the correlation only slightly (R^2 = 0.71), and did not reduce the significance of the relationship ($P<0.01$). Fitting a sigmoidal relationship increased the R^2 to 0.73; this was not a significant improvement. Although accuracy of the linear prediction is clearly reduced at the extremes of the data set, in practice N concentrations of less than 1% or above 6%

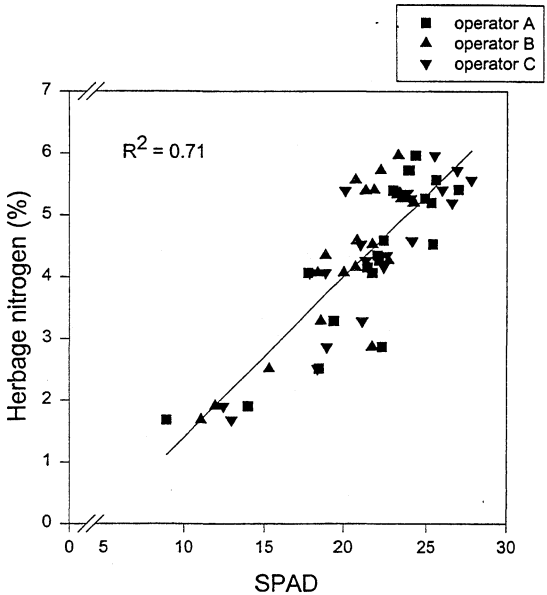

Figure 5. Relationship between SPAD and herbage N (%) for three operators.

are unlikely during spring, when the chlorophyll meter is of most use in indicating likely response to fertiliser-N.

The data indicate that the chlorophyll meter has potential for producing reliable results independent of operator (average standard deviation for each tray = 7.7%), and thus could be a useful tool for growers or field representatives. As discussed in the introduction, herbage N% is an indicator of vegetative and reproductive yield in perennial ryegrass. Thus the chlorophyll meter has potential for use in ryegrass to indicate yield.

Conclusions

The sap nitrate test, whether assessed subjectively (by eye) or objectively (using the Nitracheck meter), was not reliable as an indicator of nitrogen status in ryegrass, thus it could not be recommended for use in ryegrass without considerable further work in calibration. However, the sap nitrate test could have importance in identifying high nitrate in ryegrass prior to stock grazing. With increased use of nitrogen in pastures, and increased grazing of seed crops before 'closing', high sap nitrate could have serious effects. The relationship between protein-N and sap-N, and the limits on accumulation (e.g., due to temperature or photosynthetic rate (van Kuelen and Seligman, 1987; Millard, 1988)), require elucidation before best management practices which involve high-N use as well as grazing can be established.

The chlorophyll meter was related to herbage N% and, as readings were relatively independent of operator, has potential for use as a field tool to establish N-deficiency in ryegrass. However, factors such as leaf age, moisture status, plant population, cultivar, availability of other nutrients, season and location, can influence leaf greenness (Waskon *et al.*, 1996), as can herbicides; all of these factors must be considered when interpreting results. As a consequence, the chlorophyll meter is likely to be most useful when the only limiting factor is nitrogen availability and when the calibration for leaf greenness is for a particular stage of ontogeny for a given cultivar.

The direct relationship of the chlorophyll meter readings to yield has yet to be established, but it is related via herbage N%, and the latter has been shown to be available sufficiently early in the season to be able to modify outcomes in ryegrass seed crops (Rowarth and Archie, 1994, 1995). Furthermore, the chlorophyll meter meets criteria 3, 4 and, once the initial investment in the meter has been made, running costs are negligible. The relationship between deficiency and amount of fertiliser required has yet to be determined, as has the effect of cultivar on calibration. However, these results suggest that further research with the chlorophyll meter in perennial ryegrass is warranted.

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