THE MEASUREMENT OF NITROGEN DEFICIENCY IN GRASS SWARDS

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INTRODUCTION

Attempts to estimate the degree of nitrogen deficiency in plants have been made mostly by soil or plant analysis. Soil tests are considered to be generally unsatisfactory (Webber [8]) and at best can give information only on the supply of nitrogen at different times. On the other hand, foliar analysis, while allowing for the integrating powers of the plant, has rarely been calibrated against any more fundamental standard of deficiency than dry matter yield at, say, harvest time. The purpose of the present paper is firstly, to arrive at a more fundamental measurement of nitrogen deficiency and, then, to test possible techniques for estimating it.

The concept of nitrogen stress

Current nitrogen deficiency in plants may be defined as the extent to which nitrogen is limiting plant growth in the environmental conditions prevailing at that particular time. This specific meaning of nitrogen deficiency will be referred to as the 'nitrogen stress' in the plant.

Nitrogen stress can be expressed in terms of the potential growth response to nitrogen. To measure this at a given time it is necessary to know the growth rate of the plant under existing conditions and that of a similar plant whose nitrogen supply is non-limiting. Since

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it may often be required to compare the nitrogen stress of plants of differing size, it is more suitable to use the relative growth rate R (Blackman [3], Briggs *et al.*[4]) than the absolute growth rate. Neither of these rates is constant over the life of the plant but the former is likely to change less.

The hypothetical curves of Figure 1 show how R may respond to nitrogen supply in different environments. *
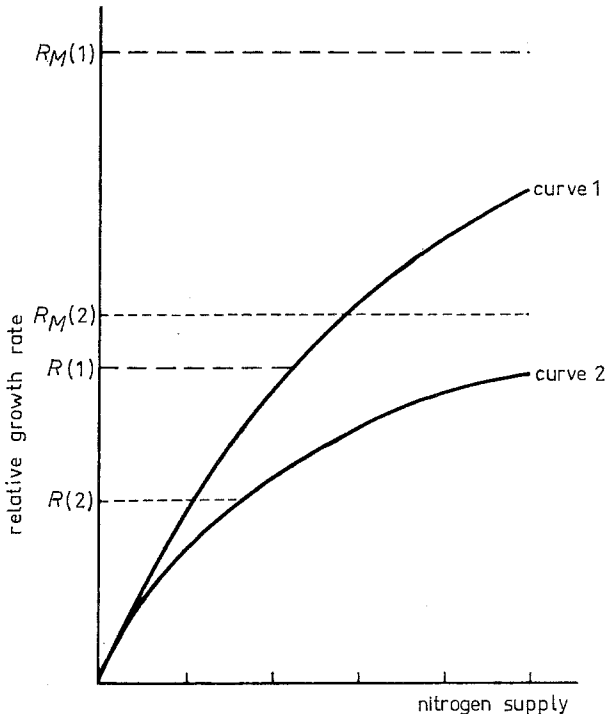

Fig. 1. Curves of hypothetical growth response to nitrogen supply; (1) favourable environment; (2) unfavourable environment. In both curves R represents 50% nitrogen stress.

Here, in the more favourable environment (Curve 1) there is, at any particular nitrogen supply, a greater response than in Curve 2 to a further increase in nitrogen supply; that is, the degree of

* The Mitscherlich equation used for these curves, implying a gradual or asymptotic approach to maximum relative growth rate, is employed only as an approximation and without any claim to its theoretical justification. It is not intended in this work to discuss toxic situations.

nitrogen stress is greater in the environment producing Curve 1. As the maximum (R_M) on either curve is approached the response to nitrogen, and thus the nitrogen stress, becomes negligible.

The degree of nitrogen stress, S_N, may be expressed as the percentage by which the relative growth rate, R, falls short of the maximum relative growth rate, R_M, attained when nitrogen is non-limiting.

$$S_N = 100 \frac{R_M - R}{R_M} \tag{1}$$

In Figure 1 the points $R(_1)$ and $R(_2)$ have been indicated corresponding with 50 per cent stress.

In practice, may be estimated either from a fitted response curve or by measuring growth rate on a nitrogen supply known to be non-limiting.

Indices of nitrogen stress

The direct measurement of nitrogen stress is laborious for routine use since each measurement of relative growth rate requires two destructive samplings at intervals of several days. If, however, an indirect measurement could be found without these disadvantages and which was a good estimator of nitrogen stress it would have considerable agronomic value. The measurement might either be an instantaneous value such as a chemical determination, or a growth response observed over a relatively short period without sacrificing plant material. Both types of measurement have been tested in the present study as possible estimators of nitrogen stress.

Chemical analyses of plants have often been used as indices of nitrogen status but have rarely been brought into relation with changes in growth rate induced by additional nitrogen supply. In foliar diagnostic work the concentration of nitrate nitrogen or total nitrogen in tissue has been most commonly used. With nitrate, results have often been conspicuously variable; this may be due to the influence of light intensity and of forms of available soil nitrogen on nitrate content. Of more importance, nitrates do not accumulate in the plant to an appreciable extent until a substantial part of its nitrogen requirement has been satisfied; only above this level does nitrate content respond markedly to nitrogen supply. This is evidenced by many published curves relating nitrate content to plant

weight (*e.g.* Ref. [5] [7]) and has been confirmed with experiments here (Greenwood, unpublished). Thus, though internal nitrate may be useful in determining a critical point above which additional nitrogen supply leads to little response, it seems unpromising as an index of the intensity of deficiency below this point, or as an estimator of S_N. On the other hand, a more elaborated non-protein nitrogen fraction such as free alpha-amino-nitrogen might vary more continuously with changes in S_N. Further elaborated fractions such as protein nitrogen, and consequently total nitrogen, may depend on nitrogen supply during the previous period, and thus tend to reflect a historical rather than a current status. In this work both total nitrogen and free alpha-amino-nitrogen were tested as indices of S_N.

In a rather labile constituent such as free amino-nitrogen, there is a chance of wide fluctuations in concentrations with weather conditions and time of day which could lead to sampling errors. Although Allen *et al.*[1] were unable to demonstrate large diurnal variation in the concentration of free amino-nitrogen in lucerne swards, it was considered advisable to confirm this with grasses. This is reported in this paper.

In the other category of nitrogen stress indices – the measurement of rapid growth response – leaf elongation seemed promising. In a grass, elongation of the young leaf is easily measured and is found to be linear over the interval between emergence of successive leaves. Preliminary work at this laboratory had shown that full response by the rate of elongation to an additional nitrogen supply was attained in sand culture within 24 hours.

Leaf elongation response was not used directly as an index of S_N but, like relative growth rate, was expressed as a percentage of the maximum elongation rate with nitrogen non-limiting, thus:

$$L_N = 100 \frac{L_M - L}{L_M} \tag{2}$$

where L_N is an index of S_N,

L is the elongation rate of emerging leaves under the nitrogen stress being measured and

L_M is the elongation rate of emerging leaves after a non-limiting dose of nitrogen fertilizer has been applied.

A satisfactory estimator of nitrogen stress must have a

relationship to it little affected by the supply of other nutrients or, within known limits, by the stage of development of the plants. The various proposed indices were accordingly tested under a wide range of nutritional conditions over several months.

<div align="center">EXPERIMENTAL</div>

Three experiments are described. In one, the importance of the weather and the time of day in affecting the concentration of free amino-nitrogen in the leaf was studied. The purpose of the second was to obtain a broad evaluation of foliar nitrogen indices of S_N over a range of nutritional conditions and the third enabled the effect of stage of development on S_N and on the relation of indices to it, to be measured.

Experiment 1

Spaced plants of *Lolium rigidum* were grown in boxes of sand 77 cm × 82 cm × 82 cm deep. The surface of the sand was marked into 10-cm squares each containing 20 plants. The swards were watered twice weekly with 12 litres of a complete nutrient solution.

Of the four boxes used in the experiment two were provided with nitrogen at the rate of 3 mM and two at the rate of 6 mM. The nitrogen was supplied in equimolar proportions of ammonium nitrate and sodium nitrate.

Seeds were sown on May 31st 1962 in the boxes in the open.

One set of harvests (winter) began on July 19, another set (spring) on August 29. In each set a dull day and a fine day were chosen as close together as the weather permitted. On each day six samples were taken, the first at sunrise and the last at sunset. Samples were analysed for total nitrogen and free α-amino nitrogen.

At each sampling time the youngest fully expanded leaf on each tiller was sampled from two randomly selected squares in each box. Material from each pair of squares was bulked.

Experiment 2

A sward of *Lolium rigidum* was sown during May 1 to 4, 1961 at the rate of 7.4 g seed per sq.m on Karrakatta Sand (Bettenay *et al.*[2]), a highly leached soil of low nutrient content on the Swan Coastal Plain near Perth.

Fertilizer treatments (Table 1) were combined in a factorial system and were assigned to plots in two randomised blocks. They may be summarized: 6 nitrogen fertilizer levels × 2 nitrogen compounds × 2 basal fertilizer levels. Fertilizers were applied twice – with the seed, and on July 3 to 6 after intense, leaching rains. Minor elements were applied as a solution at sowing.

Each plot (24 × 1.7) m was divided into sub-plots (1.8 × 1.5 m) for sampling the sward at different harvests. The sub-plots were sampled with a centrally placed quadrat (1.2 × 0.9 m). The sub-plots were taken in serial order of position to avoid mechanical damage to the sward.

TABLE 1

Fertilizer treatments		
Basal fertilizer	C_1 (g/sq.m)	C_2 (g/sq.m)
Superphosphate	5.6	11.2
Potassium sulphate. . .	5.6	11.2
Calcium carbonate . . .	5.6	11.2
Magnesium carbonate. .	2.8	5.6
Copper sulphate	0.03	0.06
Manganese sulphate . .	0.03	0.06
Boric acid.	0.06	0.11
Zinc sulphate	0.03	0.06
Ammonium molybdate .	0.003	0.007
Ferric citrate	0.03	0.06
Forms of N-fertilizer	R_0 Ammonium sulphate R_1 Ammonium nitrate	
N-levels (g/sq.m)	N_1 2.2 N_4 9.0 N_2 4.5 N_5 11.2 N_3 6.7 N_6 13.5	

TABLE 2

Sampling times and rainfall			
Harvest (t_1)	Date	Rainfall between dates (in.)	Remarks
Experiment 2			
	May 1		Seeding rate 7.4 g/sq.m and first application of fertilizers
		10.72	
	July 3		Second application of fertilizers
		4.54	
1	July 24		
		3.71	
2	Aug. 14		
		3.43	
3	Sept. 4		
Experiment 3			
	May 8		Seeding rate 3.7 g/sq.m and first application of fertilizers
		14.96	
	June 19		Second application of fertilizers
		5.97	
1	July 1		
		5.55	
2	July 22		
		2.71	
3	Aug. 12		

When nitrogen stresses were being measured (Table 2) over a 3-weekly period, t_1–t_2, a sub-plot was sampled on each plot at t_1. About 5 g fresh youngest fully expanded leaves on each filler were immediately removed from this material for chemical analysis. In order to get a representative sample of tillers, many small sub-samples of these were taken from the quadrat and these were exhaustively defoliated and the dry matter of the sward was determined from the remainder of the tops. The next two sub-plots were reserved for dry matter determinations at t_2. On one of these an extra dressing of 22.5 g N per sq.m (calcium ammonium nitrate) was applied and lightly watered in. At t_2 these two sub-plots were sampled for dry matter. Actual calculation of relative growth rates was unnecessary, since the intervals over which R and R_M were measured, were the same and a common initial sample was used: under these circumstances the expression for S_N becomes

$$S_N = 100 \cdot \frac{\ln w_M - \ln w_2}{\ln w_M - \ln w_1} \tag{3}$$

where w_1 is the initial dry weight, w_2 the final dry weight without additional nitrogen, and w_M that with non-limiting nitrogen.

All quadrats were cut with a sheep-shearing handpiece at ground level. Sampling was confined to the hours 09.30 to 12.30.

Experiment 3

A sward of *L. rigidum* was divided into 5 blocks of 6 plots. Each plot received basal fertilizers at the C_2 rate, and one of the 6 levels of ammonium nitrate used in Experiment 2 (Table 1). All fertilizers were applied twice (Table 2) at these rates.

Each plot was divided into 12 sub-plots. These were ranked visually for yield and divided into 3 groups of four on these ranks; one such quartet was then assigned at random to each of the 3 harvests periods (Table 2). Relative growth rates were thus based on comparisons between sub-plots differing initially as little as possible. Of the quartet of sub-plots assigned to a particular S_N determination, one was allotted at random for an initial harvest at t_1; at the same time two were given "non-limiting" doses of nitrogen and these, together with the fourth sub-plot, were harvested 17 days later at t_2. For the first S_N determinations the two "non-limiting" nitrogen doses were 22.5 and 28 g N per sq.m applied as a solution of ammonium nitrate and sodium nitrate in equi-molecular porportions; for subsequent periods these amounts were increased to 28 and 33.5 g N per sq.m.

The dimensions of the sub-plots and of the quadrats used for sampling them, and the harvesting techniques were the same as for Experiment 2.

Samples for chemical analyses were taken from the untreated sub-plots at t_1 and t_2. Tillers were immediately sampled at random from the harvested material and the youngest fully expanded leaf of each was taken for chemical determinations.

Chemical Analysis

Total nitrogen was determined on dry material after digestion with sulphuric and salicylic acids, selenium and potassium sulphate at room temperature prior to heating. Free alpha-amino-nitrogen was extracted from about 5 g of fresh leaves by immersion in boiling 80% ethanol for 3 minutes followed by blending and filtering. Determination was by the ninhydrin method of Yemm and Cocking [9]. As this method also includes a small proportion of compounds other than α-amino acids, the fraction will be referred to as ninhydrin-nitrogen.

The chemical values are expressed on a dry weight basis.

<div align="center">RESULTS</div>

Experiment 1

The winter harvests commenced five weeks from emergence. The difference between growth, and between free ninhydrin nitrogen
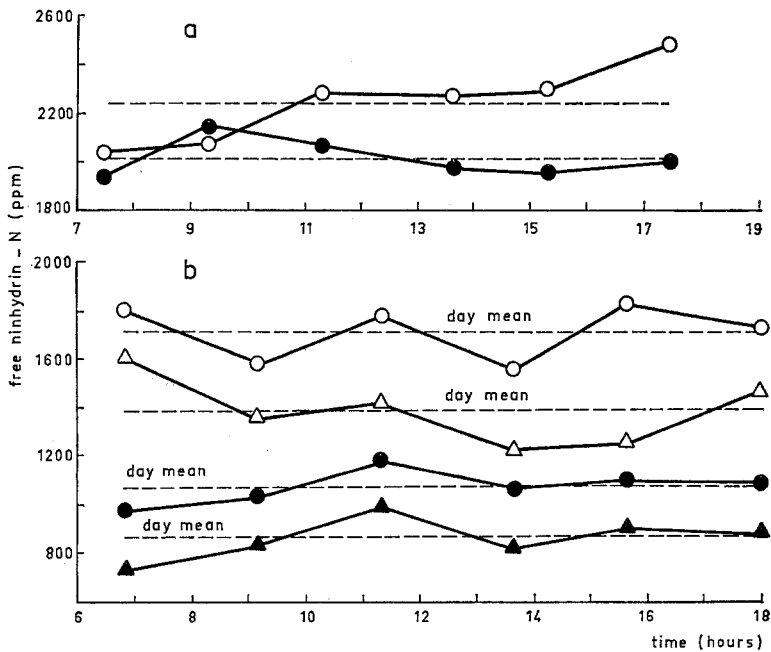

Fig. 2. Diurnal variation in free ninhydrin-N; Experiment 1. (a) Winter – ●, N_1; ○, N_2. Points are means of 4 (LSD at $P.05$ = 230 ppm). (b) Spring – ●, N_1 dull day; ▲ N_1, fine; ○, N_2, dull; △ N_2, fine. Points are means of 2 (LSD at $P.05$ = 220 ppm for N_1 and 570 ppm for N_2). *(time = time of day)* Broken lines indicate *day means*.

concentrations (Fig. 2a), on N_1 and N_2 was small, due probably to the intense leaching rainfall over this period.

At the lower nitrogen level there were no significant differences between means of concentrations either within days or between a fine and a dull day. At the higher level of nitrogen there was, again, no significant effect of weather conditions, but the concentration of free ninhydrin-nitrogen changed with time of day. Between the sampling times of 09.15 hours and 17.15 hours, which may be considered as the working day, there was an overall increase of 20 per cent.

At this time of year and early stage of plant growth, then, the concentration of free ninhydrin-nitrogen was independent of the change in solar radiation due to heavy and continuous cloud. At a daily mean of 2000 ppm (N_1) the concentration was also unaffected by time of day. At a higher level than this there was a small significant increase over the working day.

In spring the concentrations of free ninhydrin-nitrogen were consistently and significantly higher on the dull day at both N_1 and N_2 (Fig. 2b). This was probably due to the rapid increase in physiological age of plants at that time rather than to weather conditions, since a week had elapsed before fine weather returned after the initial harvest. This interpretation is supported by the fact that total nitrogen concentration (unlikely to be affected by the weather) was also higher on the dull day. Furthermore, if the level of radiation had influenced the concentration of free ninhydrin-nitrogen, then it would show as an interaction between time of day and weather conditions. Analysis of variance showed no such interaction.

The overall effect of time of day on the concentration of free ninhydrin nitrogen was not significant although a small rise occurred in the N_1 plants between sunrise and 11.30 hours ($p = .02$ for the fine day and $p = .08$ for the dull day).

Experiment 2

In general the swards on higher levels of nitrogen produced more dry matter (Table 3), higher concentrations of leaf nitrogen and smaller stresses than on lower levels.

Larger yields were produced with C_2 than C_1 level of basal fertilizer (Table 3). But because current values of S_N and leaf nitrogen were almost unaffected by the level of C, it is concluded that its direct

influence on yield must have been early and was eventually reduced by leaching.

TABLE 3

Dry-matter yield (g/sq.m) of *Lolium rigidum* swards: Experiment 2. C_1 = low basal and C_2 = high basal fertilizer; R_0 = ammonium sulphate and R_1 = ammonium nitrate. Values are means of 2

N-supply	C_1R_0	C_1R_1	C_2R_0	C_2R_1
Harvest interval 1–2				
1	45	38	56	50
2	59	62	74	62
3	87	74	91	87
4	80	78	142	131
5	84	78	142	123
6	106	102	152	131
Harvest interval 2–3				
1	99	68	128	98
2	186	154	182	176
3	169	167	256	239
4	228	166	323	258
5	257	226	315	332
6	284	219	390	296

Plants receiving ammonium sulphate had higher yields of dry matter (Table 3), higher leaf nitrogen and lower stresses (Fig. 3) than those with ammonium nitrate, indicating that ammonium ions were leached less extensively than nitrate ions.

Leaf concentrations of total nitrogen and free ninhydrin-nitrogen were much lower during the second harvest interval than the first.

The intensity of the winter rainfall (Table 2) caused rapid leaching of nitrogen from the root zone. Consequently leaf nitrogen values fell rapidly over the 3-weekly period used for the estimation of relative growth rate and it was likely that the average of the initial and final leaf nitrogen values would reflect stress more faithfully than would either of these values separately. Accordingly these average values of leaf nitrogen were used in Figure 3.

Three forms of curve (Fig. 3) were fitted by the method of least squares to the relationship between S_N and leaf nitrogen (x) to seek the most suitable model for evaluating treatment effects.
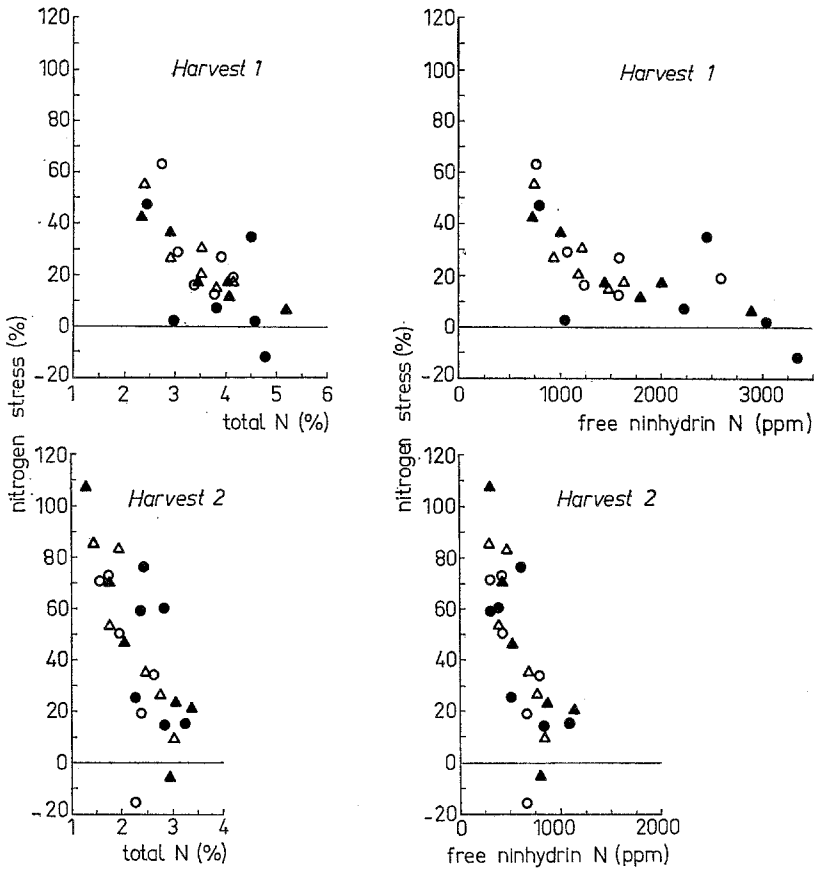
$$\text{(i) } S_N = a + bx \tag{4}$$

Fig. 3. The relationships between nitrogen stress and total N, and between nitrogen stress and free ninhydrin-N in the leaf: Experiment 2
● low basal fertilizer with ammonium sulphate (C_1R_0)
○ low basal fertilizer with ammonium nitrate (C_1R_1)
▲ high basal fertilizer with ammonium sulphate (C_2R_0)
△ high basal fertilizer with ammonium nitrate (C_2R_1)
    Values are means of two.

where x is leaf nitrogen concentration

$$\text{(ii)} \quad S_N = a + bx + cx^2 \tag{5}$$

$$\text{(iii)} \quad S_N = \frac{b}{x - x_0} \tag{6}$$

where x_0 is the leaf nitrogen concentration corresponding with zero growth.

The rectangular hyperbola (iii) was selected as a curve of equal mathematical simplicity to (i), but with the biological advantage that it is asymptotic to the x-axis.

Separate curves were fitted to the values for the suite of 6 nitrogen levels for each combination of basal fertilizer (C) form of nitrogen (R) treatment, harvest interval and leaf nitrogen fraction.

In only 2 out of the 16 cases were the quadratic regressions significantly better than the linear. The fitting of a third parameter accordingly seemed unjustified and attention was concentrated on the two two-parameter curves.

For any one harvest interval and leaf nitrogen fraction, the curves for the four CR treatments did not differ significantly, whether linear or hyperbolic; combined linear and combined hyperbolic curves were therefore fitted to the data of Figure 3.

These combined regressions are highly significant ($P < .001$; 22 d.f.); the difference between corresponding linear and hyperbolic deviation mean squares is not significant.

A comparison of residual variances associated with the curves of Figure 3 (Table 4) indicates that, whether a linear or hyperbolic model is used, ninhydrin-nitrogen does not predict S_N significantly better than total nitrogen.

TABLE 4

Comparison of leaf indices as predictors of nitrogen stress					
(a) Experiment 2					
Harvest interval	Total variance of stress (23 d.f.)	Variance of deviations (22 d.f.) from regression of stress on			
		Total N		Free ninhydrin-N	
		Linear	Hyperbolic	Linear	Hyperbolic
1–2	311	163	155	171	120
2–3	1127	415	450	456	406
(b) Experiment 3					
Harvest	Variance of Stress (5 d.f.)	Variance of deviations (4 d.f.) from regression of stress on			
		Total N	Free ninhydrin-N	L_N	
1	158	24	27	10	
2	105	66	59	23	

Experiment 3

The higher of the two 'non-limiting' nitrogen levels used never produced yields significantly greater than the lower, their mean

yield was therefore considered as the maximum for the calculation of R_M.
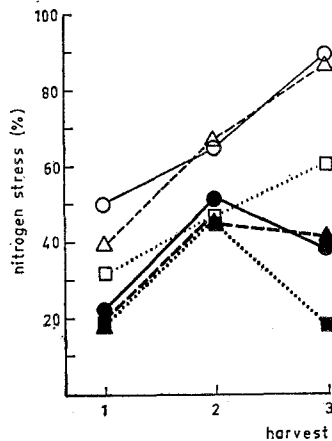

Fig. 4. Changes of nitrogen stress in an uncut sward of *L. rigidum* on a leached soil following recent application of nitrogen fertilizer (g N/sq.m): ○, 2.2; △, 4.5; □, 6.7; ●, 9.0; ▲, 12.4; ■ 16.8 (Experiment 3).

The wide range of nitrogen fertilizer rates applied at seeding produced a range of stresses from 19–50% at the first measurement (Fig. 4) and these increased until the second measurement. After Harvest 2, swards of the higher nitrogen treatments were very dense (*ca* 200–300 g /sq.m dry matter), they commenced to lodge and stresses fell. However, stresses on lower nitrogen swards continued to rise. On the lowest level (2.25 g N/sq.m) nitrogen stress rose from 50% to 90% between first and last measurements.

As in Experiment 2, chemical determinations were made on material harvested at both t_1 and at t_2, and the average of these values was used in the computation of regressions (Fig. 5).

The relationship between S_N and its indices (Fig. 5) appears relatively stable until after Harvest 2, but thereafter, with the appearance of the inflorescence (Harvest 3), internal factors have become so strong that a comparison with the earlier harvests cannot be considered. Consequently, statistical comparisons between times and between indices were confined to the first two harvests.

Linear regressions only were fitted to the data of Figure 5 as the range of stresses at any one time was too limited to elaborate on
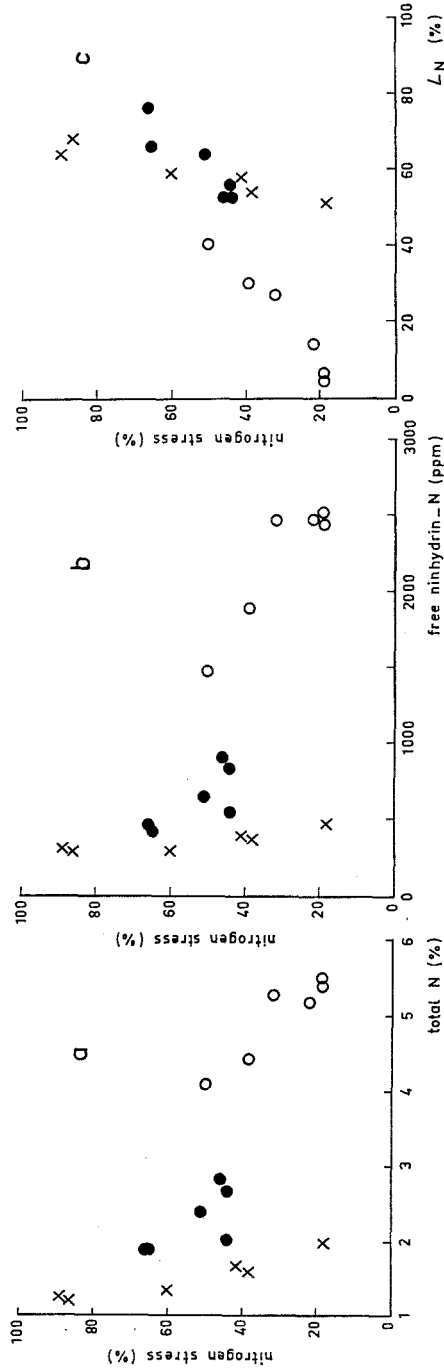
Fig. 5. The relationship between nitrogen stress and (a) total N, (b) free ninhydrin-N and (c) L_N in Experiment 3: ○ Harvest 1; ● Harvest 2 and × Harvest 3.

models. Separate regressions were fitted for each harvest and each index of S_N.

At Harvest 1, S_N gave highly significant regressions on all three indices (total N, free ninhydrin-N and L_N) but at Harvest 2, only that on L_N was significant.

Linear regressions were then fitted to the combined data of Harvests 1 and 2 for each index. From this it was determined that for a given index the regression slopes of the separate harvests did not differ significantly from the common slope. However the regressions showed lateral displacement at the 5% significance level for total nitrogen and L_N and at the 10% level for ninhydrin nitrogen.

Comparison of the residual variance of the separate regressions (Table 4b) shows, as in Experiment 2, that free ninhydrin-nitrogen was no better a statistical predictor of S_N than total nitrogen. However, L_N was consistently though not significantly a better index than foliar nitrogen. It was also the only index to give a significant linear regression on S_N at Harvest 2.

<div align="center">DISCUSSION</div>

Nitrogen stress

It is clear that nitrogen stress cannot be a substitute for a complete specification of the surface relating yield responses to nitrogen supply and to all other factors affecting it; such a complete specification would be required to enable the economics of fertilizer application to be appraised with precision. As a single value characterizing the surface, however, nitrogen stress has much to commend it. It enables the importance of nitrogen supply as a limiting factor in the ecosystem to be assessed, and this may assist in the interpretation of experiments. Furthermore, unlike measurements based on slope, it can be estimated without invoking any assumptions as to the form of the response surface; it requires only values for the maximum (R_M) and actual (R) relative growth rates. Since the response curve (*i.e.* the section of the response surface at prevailing levels of factors other than nitrogen supply) generally has an extensive plateau around the optimum, high precision in the choice of nitrogen levels to determine R_M is unimportant.

Relative growth rate (and hence nitrogen stress) has in principle

an instantaneous value. In practice, however, it can only be measure over a period of several days, although it has been found in this laboratory (Greenwood, unpublished) that the full response to a non-limiting dose of nitrogen can be achieved within a day. Extending the measurements of relative growth rate over 17 or 21 days, as in the work described here, obscures this rapid response by allowing cumulative effects on the plant and possible soil nitrogen changes (leaching or mineralization) to affect the determination. On the other hand, a very short period of measurement – 24 hours, for example – has the disadvantage of permitting temporary weather conditions to influence the results. A period of several days would enable weather fluctuations to be averaged, and the degree of replication required to be reduced to a manageable figure. Two weeks is suggested as a suitable compromise.

It has long been realised that nitrogen fertilizers leach rapidly from the root zone in most soils of the Swan Coastal Plain, and the data from Figure 4 demonstrate the severity of this. If an arbitrary value of 25 per cent is taken as an acceptable maximum nitrogen stress in a sward, then Figure 4 shows that two applications of 16.6 g N per sq.m were insufficient. Furthermore, after two applications of 2.25 g N per sq.m the sward remained so deficient in nitrogen that this element alone limited growth by never less than 50 per cent and, at the end of winter, by 90 per cent.

Indices of nitrogen stress

Nitrogen stress may be measured directly at any time in the life of the sward when growth is possible; the use of the three indirect indices is, however, somewhat more limited for their value depends on a reasonable degree of constancy in the relationship between them and nitrogen stress, in the face of advancing development and changing conditions. Between Harvests 1 and 2 the regressions underwent a lateral shift, but the slopes did not change; it seems probable, too, that the regressions would be reasonably constant prior to Harvest 1, except perhaps for the short period between emergence of the seedlings and depletion of seed reserves. Between Harvests 2 and 3, on the other hand, regression slopes show a marked change which coincides with the emergence of the inflorescence and places an obvious time limit on the use of any of the three indices studied. Unless correction can be made for the

influence of internal plant factors, then, the reliable use of indices must be limited to some 10 weeks after emergence.

The accumulation of cell-wall material in grasses proceeds slowly until the approach of flowering, when it accelerates rapidly, and this has a marked effect on values for nitrogen fractions expressed on a dry-weight basis. It may be argued that if chemical indices of S_N were expressed on a fresh-weight basis then the period of constant regression could be extended. Some evidence for this may be gained, for instance, by converting the data for free ninhydrin nitrogen concentration in Experiment 3 to a fresh-weight basis. Expressing this in terms of Figure 5: $S_N = 50\%$ corresponds with 1400 ppm ninhydrin nitrogen at harvest 1, 700 at Harvest 2, and 350 at Harvest 3 – a decrease by a factor of 0.5 at each harvest. The use of fresh-weight data brings these values to 200, 125, and 75 ppm respectively – a factor of about 0.6 at each harvest. The expression of foliar nitrogen on a fresh-weight basis, then, reduces but does not completely account for the effect of internal factors, and so would not substantially prolong the period of a constant relationship with S_N.

The curves relating nitrogen stress to the foliar nitrogen indices seem to be little affected by other nutritional conditions. Within the limits of the precision of Experiment 2, both for total nitrogen and free ninhydrin nitrogen, the same estimation curves for S_N can be used irrespective of whether the source of nitrogen was ammonium sulphate or ammonium nitrate, or whether for example, 0.55 or 1.1 g N per sq.m of superphosphate or potash were applied. However, in Experiment 2, the reduction in basal fertilizers (C_1) produced only balanced and subclinical deficiences and it is possible that more severe or unbalanced deficiencies might influence the relationship. It is likely (Possingham [6]) that molybdenum deficiency would upset the relationship with ninhydrin nitrogen.

Total nitrogen concentrations may be expected to be less sensitive to fluctuations in nitrogen stress than free ninhydrin nitrogen, since the latter is labile. That total nitrogen did not prove to be less sensitive in this work, despite the intense changes in S_N with leaching, is due to the choice of a suitable organ for chemical analysis. The leaf harvested had only just stopped growing and hence its nitrogen concentration would be strongly influenced by the prevailing degree of nitrogen stress. Total nitrogen and free ninhydrin nitrogen concentrations in the youngest fully expanded leaf appear

to have equal predictive value for nitrogen stress; since determination of ninhydrin nitrogen is simpler and more rapid,* this index seems preferable. It can be concluded from Experiment 1 that the concentration of this fraction will not be greatly influenced by the time of day when sampling is likely to be performed, nor by the weather. This is an advantage over the use of nitrate-nitrogen.

In practice, the use of nitrogen content as an index of instantaneous stress requires only a single determination. If in Experiments 2 and 3 the nitrogen status of the unsupplemented plants had remained constant through the period t_1 to t_2, the value at t_1 alone would have been needed and in this event a closer relationship with nitrogen response would probably have been found.

With L_N as the index of nitrogen stress, it is clear that the curve for Harvest 1 (Fig. 5) intersects the Y-axis at a value of nitrogen stress between 10 and 20 per cent – in other words, leaf extension is maximal even where the relative growth rate of the plant could be increased by additional nitrogen. Consequently, at this early stage of development L_N could not be used as an index of *low* nitrogen stresses. Over the limited range of nitrogen stresses at Harvest 2, the curve seems approximately linear, and the same is true at Harvest 3, with the surprising addition that the extrapolated curve cuts the X-axis at about 45 per cent. If this extrapolation is justified, it implies that leaf elongation responds markedly to added nitrogen where the relative growth rate of the whole plant does not. Moreover, differences in nitrogen stress from 20 to 90 per cent correspond with only a very small change in the response of leaf elongation to nitrogen. L_N then is an insensitive index of nitrogen stress at Harvest 3.

Since the recycling of nitrogen within the plant permits a limited rate of leaf elongation even where nitrogen supplies are inadequate for any dry weight gain, it is to be expected that a nitrogen stress of 100 per cent would correspond with an L_N value somewhat below this figure. The data available unfortunately do not cover a wide enough range to enable this expectation to be tested.

Despite its unsuitability for very low nitrogen stresses, L_N seems

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* Ninhydrin nitrogen can be accurately determined 5 to 10 times as rapidly as total nitrogen using standard equipment no more expensive than that required for a micro-kjeldahl determination.

a very promising index for practical use. It is no more time-consuming than a foliar nitrogen determination, and requires neither apparatus nor technical skill. As is shown by the figures of Table 4, it also has promise of greater precision.

This paper has served to introduce the concept of nitrogen stress, to give a first indication of how it may vary in plants of one species grown under a wide variety of nutritional conditions and almost through their entire life cycle, and to suggest some indirect indices of it which could be of value in practical agronomy. Future work can clearly be limited to the part of development preceding flowering, and there seems at present little need to vary the type of observation from those which have proved successful as indirect indices. Attention must now be directed to the derivation of reliable calibration curves for the most useful indices; further studies of the constancy of these calibration curves when the plants are subject to a variety of other nutrient treatments or to defoliations; and the extension of the methods to cereals and other grass species.

SUMMARY

A new expression for the degree of nitrogen deficiency is proposed. *Nitrogen stress* is defined as the percentage shortfall of relative growth rate (R) of a plant below that which the same plant might attain in the absence of any limitation due to nitrogen supply (R_M), other conditions remaining unchanged that is,

$$S_N = 100 \frac{R_M - R}{R_M}.$$

Nitrogen stress, was measured in the field in uncut swards of *Lolium rigidum* at different times, with varied levels of basal fertilizers and differing forms of nitrogen supply.

Because the measurement of S_N is laborious, indirect indices of it were sought. The chemical indices tested were total nitrogen and free ninhydrin-nitrogen concentrations in the newly mature leaf. A rapid growth index

$$L_N = 100 \frac{L_M - L}{L_M}$$

where L is leaf elongation rate and L_M is the elongation rate after a non-limiting dose of nitrogen, was also tested.

S_N can be estimated reliably by the three indices studied over the whole range of experimental treatments employed, and up to about 10 weeks following the emergence of the seedlings. L_N appears to be the best of three.

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