Plant and Soil XXIV, no. 2 April 1966

NITROGEN STRESS IN WHEAT - ITS MEASUREMENT AND RELATION TO LEAF NITROGEN

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INTRODUCTION

The current severity of nitrogen deficiency in a crop may be measured by the short-term increase in its relative growth rate which a non-limiting supply of nitrogen induces. Greenwood *et al.*[2] used the term 'nitrogen stress' for this meaning of nitrogen deficiency and defined it as $100\ (R_M - R)/R_M$. Here, R is the relative growth rate of the crop on one half of a split plot measured over a short interval of time, t_1 to t_2, and R_M is the value of R attained when the non-limiting supply of nitrogen is applied at t_1 to the other half of the plot. Relative growth rate is given by the expression $(\log_e w_2 - \log_e w_1)/(t_2 - t_1)$ where w_1 and w_2 are the dry weights at t_1 and t_2.

In two field experiments it was shown [2] that the two levels of a complete basal fertilizer and the form of nitrogen used did not significantly influence the relation of nitrogen stress to concentrations of leaf nitrogen fractions in *Lolium rigidum*.

In this paper nitrogen stress is studied in the young wheat plant. The characteristics of changes in relative growth rate and in concentrations of leaf nitrogen fractions with increasing stress are measured over one set of conditions but with the greater precision given by pot culture compared with field plots. The ability of total nitrogen and of free ninhydrin nitrogen concentrations to estimate nitrogen stress is measured with a view to using these fractions as predictors.

EXPERIMENTAL

Germinated seeds of the Gabo variety of wheat were sown in free-draining pots, which contained 14 kg of a nitrogen-free quartz sand. Seedlings emerged on 24th March 1964 and were thinned at random to 19 per pot.

One litre of the following nutrient solution, made up in tap water, was added to each pot twice weekly from emergence (values are the concentrations in mM): calcium chloride, 1; sodium dihydrogen orthophosphate, 0.33; magnesium sulphate, 0.75; potassium chloride, 1; ferric citrate, 1.8×10^{-3}; copper sulphate, 5×10^{-4}; manganese sulphate, $5 \times 10\ ^{3}$; boric acid, 1.5×10^{-2}; zinc sulphate, 1.5×10^{-4}; ammonium molybdate, 1×10^{-5}. Nitrogen, in varying concentrations ranging from 0.125 to 10 mM (Table 1), was supplied as an equi-molar solution of ammonium nitrate and sodium nitrate.

An estimation of the nitrogen stress for plants on each level of supply was obtained by providing 4 adjacent pots, of which 2 were reserved at random for w_1 and w_2 measurements, and 2 for additional nitrogen, applied at t_1, for the estimation of R_M. These 'non-limiting' nitrogen levels were 10.00 and 12.00 mM.

The experiment was arranged in 7 randomized blocks.

The harvest interval (t_1 to t_2) was 2 weeks and commenced after the 3rd week from emergence.

Relative growth rates were based on the dry weight of tops severed at the junction with the root.

At t_1, the youngest fully expanded leaf on each tiller was removed for chemical analysis from those plants being harvested for w_1, and at t_2 the procedure was repeated on the w_2 plants. Sub-samples of the youngest fully expanded leaves were extracted with ethanol and analysed chemically according to procedures described by Greenwood *et al.*[2].

All chemical values presented are based on dry weight.

RESULTS

All levels of supplied nitrogen increased relative growth rate between the third and fifth week (Table 1, Fig. 1b), so the negative slopes of the dry-matter yield curves reflect a depression of growth rate prior to this period, (Fig. 1a).

Nitrogen stresses ranged from about zero, in those plants with the highest nitrogen supply, to 83 per cent where deficiency was greatest (Table 1, Fig. 1c).

The dry weights at t_2 of plants given the 'non-limiting' level of 10 mM did not differ significantly from those given 12 mM; accordingly R_M was calculated from their means.

TABLE 1

The effect of nitrogen supply on relative growth rates (R and R_M), nitrogen stress, and leaf nitrogen in the young wheat plant
(Values are means of 7 pots of 19 plants each; chemical values are on a dry-matter basis; t_1 = 3 weeks, and t_2 = 5 weeks, from emergence)

N-supply level m*M*	R %/day	R_M %/day	N-stress %	Total N %		Soluble N %		Free ninhydrin N (ppm)	
				t_1	t_2	t_1	t_2	t_1	t_2
0.125 *	2.17	12.53	83	2.15	2.08	0.22	0.20	710	730
0.25 *	4.03	12.95	69	2.58	2.44	0.34	0.26	890	710
0.50 *	5.54	13.53	60	3.06	2.30	0.31	0.27	950	700
1.00 *	7.07	12.53	44	3.88	2.55	0.41	0.31	1320	790
2.00	8.82	11.63	24	4.78	2.85	0.63	0.34	1910	950
4.00	10.27	11.45	10	6.19	3.68	0.89	0.37	2500	1120
8.00	11.02	11.08	1	7.10	5.18	1.31	0.72	3870	2100
10.00	11.27	11.57	3	7.17	5.79	1.48	0.82	4680	2590

* Symptoms of nitrogen deficiency produced at these levels.

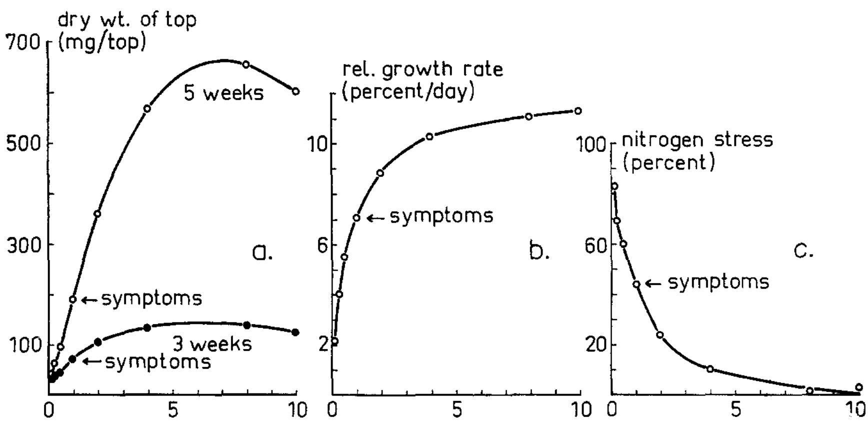

Fig. 1. Effect of N-supply on (a) dry matter, (b) relative growth rate of tops between the 3rd and 5th week, and (c) nitrogen stress (means of 7 pots of 19 plants).

Symptoms of nitrogen deficiency appeared on plants with a relative growth rate of less than 8 per cent per day. The highest rate achieved with a constant nitrogen supply was just over 11 per cent per day, although some R_M values obtained from plants initially on a low nitrogen supply were as high as 13 per cent per day (Table 1). This higher growth rate ($P < 0.001$) may have been due to the greater concentration of other nutrients in these juvenile, nitrogen deficient plants at t_1.

The relation of nitrogen stress to nitrogen supply is curvilinear (Fig. 1c); the curve cuts the ordinate at about 100 per cent stress. In this experiment, zero stress is attained at a concentration of 10 mM of supplied nitrogen, a level at which no further increase in growth rate could be achieved by adding more nitrogen.

Symptoms of nitrogen deficiency appeared in all plants with stresses greater than 40 per cent.

The negative and slightly curvilinear slope relating relative growth rate and nitrogen stress (Fig. 2) can be extrapolated to

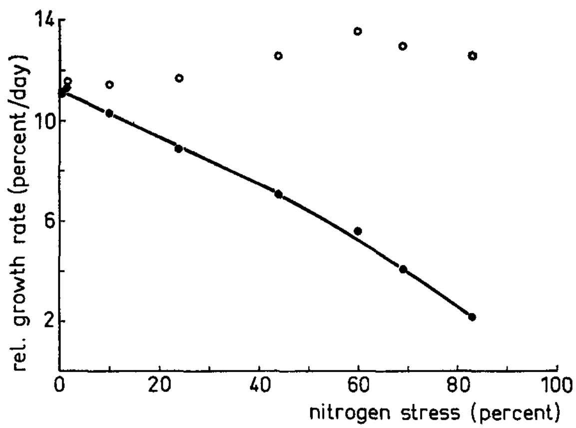

Fig. 2. Relative growth rate and nitrogen stress • R, ○ R_M (means of 7 pots of 19 plants).

nitrogen stress = 100 per cent for relative growth rate = 0 in accordance with theoretical expectation. The curvature is due to R_M values being significantly higher ($P < 0.001$) for the more deficient plants.

Concentrations of leaf nitrogen fractions fell substantially during the 14-day harvest interval (Table 1); this effect was greater at the higher concentrations. A chemical value expressing the average concentration in the plant over this time is required here in order to be related to the average nitrogen stress between t_1 and t_2. As the characteristics for the time-changes in concentrations cannot be described from two points in time, an appropriate mean cannot be rigorously derived from Table 1, as is done for nitrogen stress based on relative growth rate. In order to overcome this problem in a simple way, dual curves are presented in Figures 3 and 4; the concentrations at t_1 give an estimate of the ability of each nitrogen fraction to predict stress, while the arithmetic mean of the values at t_1 and t_2 provides a sounder basis for calibration against stress than either of these values separately.

The relation of nitrogen stress to the concentration of total nitrogen in the youngest expanded leaf appears linear for stresses greater than 25 per cent (Fig. 3). Below this value there is a severe

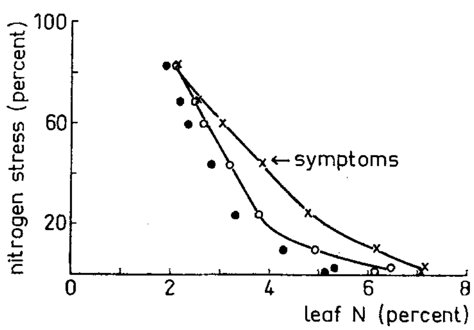

Fig. 3. The relation of nitrogen stress to total nitrogen and insoluble nitrogen of the youngest fully expanded leaf of the tiller: o total N and • insoluble N derived from the arithmetic mean of the values at the 3rd and 5th week; × total N at the 3rd week (values are means of 7 pots of 19 plants).

departure from linearity. The alcohol insoluble nitrogen fraction follows a relationship with nitrogen stress closely parallel with the relationship of stress with total nitrogen, (Table 1).

Symptoms of nitrogen deficiency occurred on all plants which showed a concentration of total nitrogen in the youngest fully expanded leaf of less than 3.9 per cent at 3 weeks, or of 2.6 per cent at 5 weeks, after emergence (Table 1). From Figure 3 a value of about 1.6 per cent nitrogen at 100 per cent stress and 7 per cent nitrogen for the absence of stress at an average age of 4 weeks might be expected.

The relation between nitrogen stress and the concentration of free ninhydrin nitrogen in the leaf (Fig. 4) is curvilinear throughout. It is probable that at a nitrogen stress of 100 per cent there remains

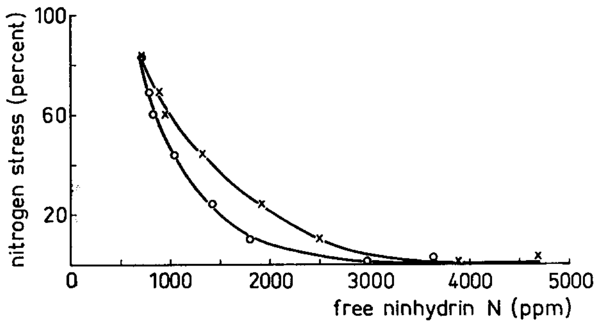

Fig. 4. Relation of nitrogen stress to free ninhydrin-N of the youngest fully expanded leaf of the tiller: × at the 3rd week, o arithmetic mean of the value at the 3rd and 5th week (means of 7 pots of 19 plants).

a pool of about 600 ppm free ninhydrin nitrogen existing in the youngest expanded leaf of Gabo wheat, even though growth in terms of dry matter would be prevented by nitrogen deficiency.

Symptoms of nitrogen deficiency appeared in plants with a concentration of free ninhydrin nitrogen in the leaf of less than 1400 and 800 ppm at 3 and 5 weeks respectively. A value of 3000 ppm may be taken as indicating the absence of nitrogen stress at 4 weeks.

For the comparison of total nitrogen with free ninhydrin nitrogen as an estimator of nitrogen stress it is necessary to fit models to Figures 3 and 4 and compare their residual variances. To simplify this a linear model was fitted to the components of the 5 means with the highest stress values and their associated total nitrogen values, and a quadratic model was fitted to the corresponding data for free ninhydrin nitrogen. There was no significant difference between the residual variances of these two estimators.

DISCUSSION

The technique used in this work permits an independent measurement of nitrogen stress to be made at each initial level of nitrogen. A very large number of pots or plots is necessary to achieve this, so that few treatments can be prescribed for one experiment – a disadvantage when it is desired to test the constancy of a relationship comprehensively. However, where treatments influence the size of plants, as in this experiment, it is essential to avoid any bias in growth response caused by associated changes in competition for light or for nutrients. For example, here, R_M values fell by over 2 per cent per day ($P < 0.001$) with increasing plant size (Table 1, Fig. 2).

The highest value for nitrogen stress, 83 per cent, was obtained with those plants receiving solutions containing 0.125 m*M* nitrogen twice weekly from emergence. Even if no nitrogen had been supplied to these plants, stresses would not have reached 100 per cent (Fig. 1) because of the influence of seed nitrogen at this early age.

The results in Table 1 show that even though no symptoms occur, growth may be limited by insufficient nitrogen by as much as 40 per cent. Further, when symptoms appear, the growth of the crop may be a very long way from being completely limited by nitrogen deficiency.

This investigation attempts to provide a calibration of nitrogen deficiency in the wheat plant in terms of the concentration of total nitrogen, soluble nitrogen or free ninhydrin nitrogen, in the youngest expanded leaf of the tiller. It can be argued, *a priori*, and it has also been demonstrated in work to be published subsequently, that nitrogen stress tends to increase in plants given a constant supply of nitrogen in a steady environment. It can be taken, then, that over the interval t_1 to t_2, nitrogen stress was increasing. For the purpose of calibration, the curves derived from the arithmetic means of concentrations at t_1 and t_2 may be used as a first approximation. Again, later work with another species suggests that the arithmetic mean over-estimates the concentration value occurring mid-way in time, but that the extent of this over-estimation varies considerably with the level of nitrogen stress. This also implies that t_1 values alone are suitable only for an instantaneous prediction of stress and that if it had been possible in this work to take a shorter time interval than 2 weeks, the curves would be displaced upwards.

From the work with Lolium [2] it is likely that these curves will hold over a range of nutritional conditions and over a term a little longer than 3 to 5 weeks.

Other considerations limit the period of sampling to about 3 to 8 weeks after emergence. Prior to 3 weeks, seedlings do not tiller and it is probable that during the first 2 weeks seed reserves of nitrogen would reduce stress under all conditions sufficiently to give a false prediction of the stresses which might occur when the plant was more independent of seed nitrogen. It is also probable that nitrogen fertilizer applied to a deficient crop close to ear emergence would have little effect on grain yield [3].

In *Lolium rigidum* the best index of stress tested was derived by replacing relative growth rate with a leaf elongation measurement (L_N), that is $L_N = 100\,(L_M - L)/L_M$ where L and L_M are the leaf elongation rates associated with R and R_M respectively. This measurement had the advantage of providing a value similar to nitrogen stress without the need for a chemical, or a lengthy relative growth rate, determination. Leaf measurements in the present experiment were taken over a 2-day interval between the 3rd and 5th weeks, but because too few leaves were sampled, means obtained were not suitable for use in calibration curves for comparison with leaf nitrogen, and so are not reported in this paper. Nevertheless, a correlation coefficient of $+0.927$ ($P < 0.001$) was obtained between nitrogen stress and L_N.

Total nitrogen and free ninhydrin nitrogen have several advantages over the more commonly used nitrate nitrogen as indices of nitrogen deficiency, *viz*

(i) they are readily measurable over the whole range of nitrogen stress (Figs. 3 and 4)
(ii) they are almost unaffected by diurnal and weather variation [1 2]
(iii) their accurate determination is simpler and, for free ninhydrin nitrogen, much more rapid
(iv) they are, to a large extent, unaffected by the composition of the nitrogen supply [2].

The following values from the arithmetic mean curves in Figures 3 and 4 are obtained by interpolation: –

Nitrogen stress %	Total nitrogen %	Free ninhydrin %	Nitrogen stress %	Total nitrogen %	Free ninhydrin nitrogen %
10	4.95	0.180	50	3.00	0.098
20	4.00	0.150	60	2.75	0.086
30	3.60	0.130	70	2.50	0.078
40	3.30	0.113	80	2.15	0.072

Nitrogen stress gives an estimate of short-term growth response at the time of measurement. It does not necessarily follow that the yield of grain will reflect this stress.

The free ninhydrin nitrogen concentration of the youngest fully expanded leaf can also be used to estimate the total nitrogen concentration of that organ (Fig. 5). The separate linear regressions

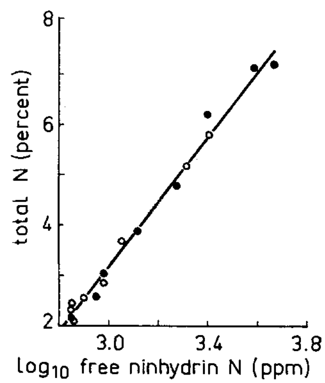

Fig. 5. Free ninhydrin-N as estimator of total N in the youngest expanded leaf • 3 weeks ○ 5 weeks at 8 levels of N (means of 7 pots).

fitted to that data for t_1 and t_2 are not significantly different, and the pooled data provide the following relationship:

Total N (per cent) $= -16.14 + 6.43 \log_{10}$ free ninhydrin nitrogen (ppm)

The correlation coefficient between the concentrations of total nitrogen and free ninhydrin nitrogen, +0.994, is highly significant.

SUMMARY

The intensity of nitrogen deficiency in the young wheat plant was measured over a range of 1 to 83 per cent of nitrogen stress. Within these limits of stress relative growth rate of tops fell from 11 to 2 per cent per day.

Symptoms of nitrogen deficiency appeared when the stress was greater than 40 per cent.

Nitrogen stress in the variety Gabo is calibrated in terms of the concentrations of total nitrogen, soluble nitrogen and free ninhydrin nitrogen of the youngest fully expanded leaf.

ACKNOWLEDGEMENTS

This experiment was carried out by Mr. Z. V. Titmanis, assisted by Miss M. M. P. McCafferty, Mr. A. Morrison and Miss P. Taylor, with great care. I wish to thank them and also Dr. D. W. Goodall of C.S.I.R.O., Division of Mathematical Statistics for consultation.

Received December 11, 1964

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