The Link Between Nitrogen Application, Concentration of Nitrogen in Herbage and Seed Quality in Perennial Ryegrass (*Lolium perenne* L.)

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ABSTRACT

A series of trials involving different rates and timing of nitrogen (N) fertiliser performed in New Zealand and Oregon, USA, provided the opportunity for investigation of the link between N application and seed vigour in perennial ryegrass (*Lolium perenne* L.) cv. Grasslands Nui, Linn and Buccaneer. Herbage N concentration was analysed 14 days after spikelet initiation and thousand seed weight (TSW) was measured at harvest. Seed lots of cv. Grasslands Nui with different TSW, seed N concentration and, hence, N content, were tested for germination, and vigour using accelerated ageing at 41 °C. Seedling weight was also determined. N applied in autumn, winter and early spring resulted in seed with a high TSW in all cultivars, and high N concentration in cv. Grasslands Nui; N applied at anthesis had no consistent effect on TSW. Herbage N concentration at 14 days after spikelet initiation was significantly related to TSW. Seed vigour (seedling weight and germination after accelerated ageing), was positively and significantly related to increasing TSW, N concentration and N content (the latter two measured 14 days after spikelet initiation). N concentration was the most important factor governing seed vigour, explaining over 90% of the variability in seed vigour under the conditions of these experiments. A TSW of over 2.35 g was associated with a germination of greater than 75% after accelerated ageing, and a spring herbage N concentration of over 3% in cv Grasslands Nui. A similar relationship was found between spring herbage N% and TSW for cv. Linn. No relationship was established for cv. Buccaneer. These results suggest that applying N in autumn, winter and spring to achieve a spring herbage N of greater than 3%, will improve seed vigour; this N application strategy has also been associated with good seed yield.

Additional index words: accelerated ageing, herbage nitrogen concentration, nitrogen content, seedling weight, seed vigour.

INTRODUCTION

For herbage species, thousand seed weight (TSW) is a seed quality factor which has been associated with increased seedling weight (Jin, Rowarth, Scott and Sedcole, 1996), vigour (Bean, 1980) and field emergence (Charlton, 1989; Rowarth and Sanders, 1996), and, hence, improved field production (Brown, 1977; Hampton, 1986). Within a seed lot, larger seeds have more carbohydrate reserves and are able to grow more rapidly. This gives a growth advantage which is confounded in the field, where bigger seedlings have a competitive advantage as they increase in leaf area, and hence potential light interception, more rapidly than small seedlings. Despite this, few species or cultivars have TSW as part of seed certification requirements. In New Zealand, cv. Grasslands Moata (*Lolium multiflorum* Lam.) is an exception (TSW must be greater than 4.0 g) but this is for cultivar purity reasons (Hampton, 1986), rather than vigour. In comparisons between seed lots, however, thousand seed weight is often a poor indicator of vigour (reviewed by Hampton 1991; 1997).

In a comparison of seeds of different nitrogen (N) concentration, but the same TSW, for laboratory germination, accelerated ageing and field emergence, seed N concentration was found to be a factor contributing to seed vigour (Bennett, Rowarth and Jin, 1998). Furthermore, re-examination of data from Ene and Bean (1975) shows that TSW was confounded with seed N concentration, i.e., as TSW increased, so did seed N%, as it did in the study by Jin *et al.* (1996). Hence it is possible that seed N content (TSW x N%) is a major factor in seed vigour.

There is a wealth of data in the literature to do with the importance of N application rate and timing on seed yield and its components. Occasionally it has been reported that late spring N has a significant effect on TSW (Nordestgaard, 1986), but in general TSW is more responsive to the total amount of N applied than timing (e.g., Rowarth *et al.*, 1998; Young, Silberstein, Chastain and Rowarth, 1998). This may be a reflection of the seed cleaning process, which removes small seed, or the fact that plants respond to limitations by producing fewer, but well-filled seeds rather than producing more, but poorly-filled seeds (Hampton, 1991). As N is frequently a major limitation in ryegrass seed crops, its availability during seed-filling may be more of a limitation than carbohydrate, assuming water supply is sufficient. There are, however, few reports where N concentration has been considered in seed quality, so no recommendations can be made on strategies for nitrogen management to achieve high quality seed.

This paper reports results from three field sites and two laboratory studies, in an attempt to elucidate the link between applied nitrogen and subsequent seed performance.

MATERIALS AND METHODS

Field trials were established between 1995 and 1997 in Canterbury and Manawatu, New Zealand, using perennial

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ryegrass cv. Grasslands Nui, and Oregon, USA using cv. Grasslands Nui and cvs. Linn and Buccaneer. Details of trial management have been presented in Rowarth, Boelt, Hampton, Marshall, Rolston, Sicard, Silberstein, Young and Sedcole (1998). Briefly, different combinations of rates and timings of N were applied to create a range of concentrations of N in herbage in early spring. In the Canterbury trials, 50 kg ha^{-1} N was also applied to two different plots at anthesis (Table 1). The measurements taken germane to the present research were herbage N concentration (Basson, 1976) (sampled 14 days after spikelet initiation) and TSW at harvest (ISTA, 1996).

Seed samples from the main Canterbury field trial (harvested in December 1997) contrasting in TSW and seed N concentration (Basson, 1976) were germinated (ISTA, 1996) and seedling dry weight (after drying for 16 hours at 65 °C) was measured at 14 days. A further series of cv. Grasslands Nui seed samples (from a lysimeter field trial described in full in Cookson, Rowarth, Cornforth and Cameron, 1999) were also analysed for TSW and seed N concentration, and then germinated before and after accelerated ageing (Hampton and Te Krony, 1995) for 72 h at 41 °C and under high humidity (DeFilippi, 1996).

Results were analysed using Minitab 10 for ANOVA, regression and correlations. A separation technique (Cate and Nelson, 1971), described in Rowarth *et al.* (1998) was used to establish critical thresholds for relationships.

RESULTS AND DISCUSSION

Nitrogen applied at anthesis had no consistent significant effect on TSW (Table 1). Although TSW did increase significantly in 1997, the effect was not apparent where N had been applied in autumn and winter as well as in spring. Linear regressions fitted to the individual response lines between TSW and herbage N concentration in cv. Grasslands Nui grown in field trials in New Zealand and Oregon produced R^2 of 0.00-0.82; the relationship for combined data gave an R^2 of 0.56 (Table 2). Similar relationships were observed in Oregon for cv. Linn (Table 3) and cv. Buccaneer (Table 4), although the significance was reduced where disease pressure affected seed yield (Young *et al.*, 1998). These results suggest that N taken into the plant early in the season is important for subsequent seed-filling, and support recent research with ^{15}N labelled fertiliser where approximately 30% of winter applied N and 15-20% of spring applied N was recovered in the seed (Cookson, Rowarth and Cameron, 2000). The supply of carbohydrate for the seed, much of which comes from the stem (Roy and Rolston, 1994), is likely to be enhanced by the maintenance of the flag leaf. In wheat (*Triticum aestivum* L.), large applications of fertiliser N result in leaves which remain greener, longer (Osman and Milthorpe, 1971; Ellen and Spiertz, 1980), possibly because plentiful N supply obviates the requirement for N to be remobilised from mature leaves (Morris and Paulsen, 1985).

Thousand seed weight of seed from the main Canterbury field trial had a significant effect on seedling weight, but accounted for only 41% of the variability in seedling weight (Table 5). In contrast, N concentration and N content accounted for 72 and 92% of this variability, respectively (Table 5). Seedling weight has been shown to be correlated with field emergence in *Lotus* spp. (Rowarth and Sanders, 1996), and post-emergence performance in ryegrass (Brown 1977). It is a relatively quick measurement to make, and for that reason may have some merit for consideration in vigour assessment, although careful standardisation of test conditions is required (Hampton and TeKrony, 1995). Increasing applied N in the lysimeter trial resulted in increased TSW, increased N concentration (%) and, hence, increased seed N content in cv. Grasslands Nui (Table 6). Germination under standard laboratory conditions was high (98% or greater); acelerated ageing (AA), followed by germination, showed that seed vigour increased with increasing N (Table 6).

Regression equations fitted to the individual responses showed that AA germination was significantly related to TSW, and that TSW explained 70% of the variability in seed vigour. N concentration and N content of the seeds, however, explained 94 and 93% of the variability in seed vigour, respectively (Table 7). This supports results from research reported by Bennett *et al.* (1998).

Plotting germination after AA against TSW indicated that 75% germination was associated with a TSW of approximately 2.35 g (Fig. 1). This TSW in cv. Grasslands Nui was associated with a herbage N concentration of 3% (Fig. 2), which has also been suggested as being an indicative figure for 85% relative seed yield (Rowarth *et al.*, 1998). In cv. Linn, a herbage N concentration of 3% (also identified as a critical threshold for yield; Young *et al.*, 1998) was associated with a TSW of 2.4 g (Fig. 3); the importance of TSW (and associated N status) in vigour of cultivars other than cv. Grasslands Nui has not yet been investigated. No relationship was identified

Table 1. The effect of nitrogen (N) applied at anthesis on thousand seed weight in perennial ryegrass cv. Grasslands Nui grown in Canterbury, New Zealand in 1996 and 1997.

Treatment[1]	Anthesis N (kg ha^{-1})	TSW (g)	
		1996	1997
0-0-150	0	2.38	2.32
0-0-150	50	2.34	2.48
50-50-150	0	2.48	2.39
50-50-150	50	2.31	2.33
LSD (P<0.05)		ns	0.15

[1] *N rates (kg ha^{-1} N) in autumn, winter and early spring (spikelet initiation).*

Table 2. The relationship between herbage nitrogen (N) concentration (%) analysed 14 days after spikelet initiation and thousand seed weight (TSW) in perennial ryegrass cv. Grasslands Nui in New Zealand (NZ) and Oregon (USA).

Location and stand age	Regression equation	R^2	P
South Island NZ[1]	TSW = 2.35 + 0.024 N%	0.00	ns
North Island NZ[1]	TSW = 2.42 + 0.024 N%	0.00	ns
USA 1st year, 1996	TSW = 2.11 + 0.116 N%	0.58	0.006
USA 1st year, 1997	TSW = 1.77 + 0.131 N%	0.82	0.000
USA 2nd year, 1997	TSW = 1.86 + 0.135 N%	0.81	0.000
USA 2nd year, 1998	TSW = 1.94 + 0.147 N%	0.52	0.011
Combined data	TSW = 2.02 + 0.108 N%	0.56	0.000

[1] *both first year crops*

Table 3. The relationship between herbage nitrogen (N) concentration (%) analysed 14 days after spikelet initiation and thousand seed weight (TSW) in perennial ryegrass cv. Linn in Oregon, USA.

Stand age	Regression equation	R^2	P
1st year, 1996	TSW = 2.12 + 0.106 N%	0.51	0.012
1st year, 1997	TSW = 1.88 + 0.143 N%	0.88	0.000
2nd year, 1997	TSW = 2.29 + 0.018 N%	0.00	ns
2nd year, 1998	TSW = 1.99 + 0.159 N%	0.69	0.00

Table 4. The relationship between herbage nitrogen (N) concentration (%) analysed 14 days after spikelet initiation and thousand seed weight (TSW) in perennial ryegrass cv. Buccaneer in Oregon, USA.

Stand age	Regression equation	R^2	P
1st year, 1996	TSW = 1.67 + 0.013 N%	0.00	ns
1st year, 1997	TSW = 1.58 + 0.083 N%	0.58	0.006
2nd year, 1997	TSW = 1.59 + 0.069 N%	0.56	0.008
2nd year, 1998	TSW = 1.59 + 0.162 N%	0.56	0.008

Table 5. Regression equations for the relationship between seedling weight and TSW, seed nitrogen (N) concentration and seed N content in cv. Grasslands Nui.

Regression equation	R^2	P
Seedling weight = 0.0165 + 0.00975 TSW	0.41	ns
Seedling weight = 0.0288 + 0.00434 N%	0.72	0.04
Seedling weight = 0.0301 + 0.165 Ncontent	0.92	0.01

Table 6. The relationship between nitrogen (N) strategy, thousand seed weight (TSW), seed N concentration, seed N content, germination and germination after accelerating ageing (AA) at 41°C in cv. Grasslands Nui.

Treatment[1]	TSW (g)	Seed N (%)	Seed N content (g/thousand seeds)	Germination (%)	
				Standard	After AA (41 °C)
0-0-0	2.26	1.24	0.028	98	49
0-50-150	2.30	1.35	0.031	99	55
50-50-150	2.35	1.49	0.035	98	79
50-100-150	2.44	1.48	0.036	98	76
50-100-200	2.43	1.52	0.037	98	86
LSD ($P< 0.05$)	0.10	0.05	0.004	ns	9.9

[1] *N rates (kg ha^{-1} N) in autumn, winter and early spring (spikelet initiation)*

Table 7. Regression equations for the relationships between germination after accelerated ageing (AA) at 41°C, and thousand seed weight (TSW), seed nitrogen (N) concentration and seed N content in cv. Grasslands Nui.

Regression equation	R^2	P
AA = 179 TSW - 352	0.70	0.048
AA = 133 N% - 119	0.94	0.004
AA = 4131 Ncontent[1] - 68.9	0.93	0.005

[1] *grams of N per thousand seeds*

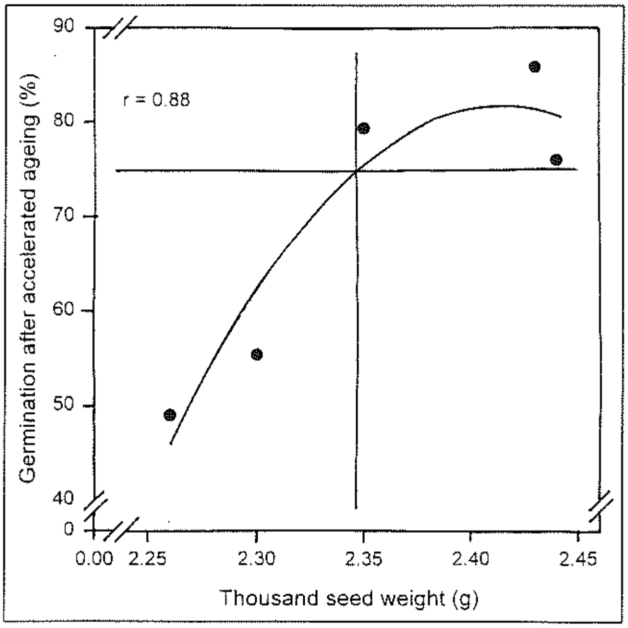

Figure 1. Relationship between thousand seed weight (g) and germination (%) after accelerated ageing at 41°C in perennial ryegrass cv. Grasslands Nui.

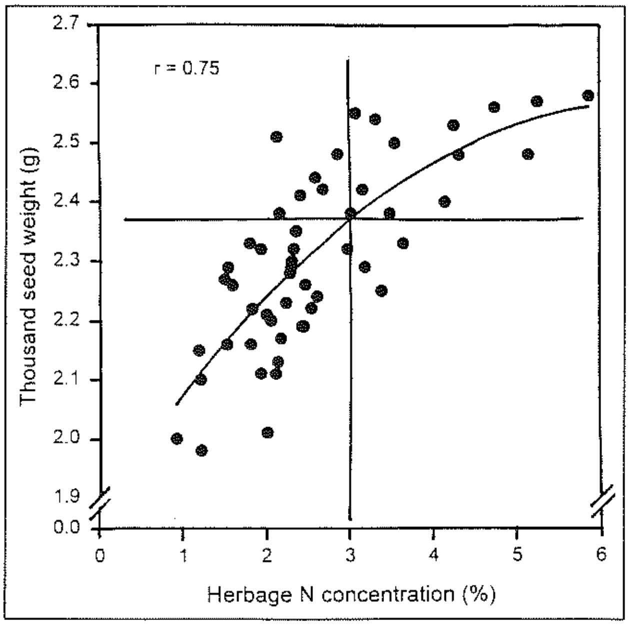

Figure 2. Relationship between herbage nitrogen concentration (%) measured 14 days after spikelet initiation and thousand seed weight (g) in perennial ryegrass cv. Grasslands Nui.

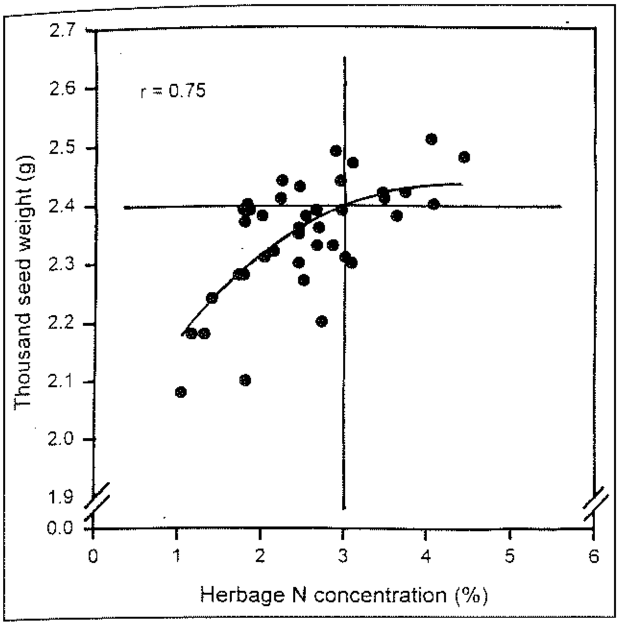

Figure 3. Relationship between herbage nitrogen concentration (%) measured 14 days after spikelet initiation and thousand seed weight (g) in perennial ryegrass cv. Linn.

for cv. Buccaneer (r = 0.32), possibly because disease pressure (Young *et al.*, 1998) resulted in small seed.

In contrast to seed yield prediction analyses (e.g., Rowarth *et al.*, 1998; Sicard and Rowarth, 1998; Young *et al.*, 1998,) prediction of TSW was not improved by considering herbage N content (dry matter yield x N concentration; data not presented) rather than herbage N concentration. Herbage N content is related to seed yield because dry matter indicates potential heads and heads are a major determinant of potential seed yield, (e.g., Cookson *et al.*, 2000), within the range appropriate for the cultivar (Hampton and Fairey, 1997). In contrast, herbage N concentration is an indication of potential stores of N which can then be used to provision the seed. Clearly, both are important for seed yield and quality, and can be achieved when N fertiliser is applied to match plant requirements (Cookson *et al.*, 1999).

CONCLUSIONS

Although the final determinant of TSW is likely to be moisture availability and length of duration of seed fill, N applied at spikelet initiation is likely to assist in giving the plant potential for high TSW, probably by allowing storage of N and by prolonging the life of the flag leaf; the latter prolongs photosynthetic acquisition of carbohydrate. Seeds with relatively large stores of both carbohydrate and protein appear to have higher vigour, and produce large seedlings which are likely to have a better chance of establishing in the field.

High vigour seed was achieved when N was applied to match plant requirements (autumn, winter and early spring). This strategy has also been shown to maximise seed yields and efficiency of nitrogen and water use (Cookson *et al.*, 1999).

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