The Relationship Between Applied Nitrogen, Nitrogen Concentration in Herbage and Seed Yield in Ryegrass (*Lolium* spp.). V. Cultivars in Chile.

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ABSTRACT

The relationships between applied nitrogen (N), chlorophyll concentration (SPAD), concentration of N (%) in herbage 21 days after spring N application, and seed yield of Italian ryegrass (*Lolium multiflorum* Lam.) cv. Concord and Montblanc, and perennial ryegrass (*L. perenne* L.) cv. Revielle and Top Hat, were tested on two soil types (an Andisol and an Ultisol) in Chile. Nitrogen treatments (50 kg ha^{-1} N in mid July, followed by 0, 50 or 100 kg ha^{-1} N at the end of September) were applied to create a range of herbage N concentrations. Chlorophyll concentration (SPAD) and combined N in herbage were analysed 21 days after the September N application. A further 0, 50 or 100 kg ha^{-1} N was applied at the end of October. Seed yield and thousand seed weight (TSW) were measured at harvest. In conjunction with 50 kg ha^{-1} N in July, 50 kg ha^{-1} N in September on the Andisol resulted in a significant increase in seed yield of cv. Montblanc, Revielle and Top Hat. On the Ultisol, seed yield of all cultivars responded significantly to 100 kg ha^{-1} N in September. Nitrogen applied at the end of October had no positive effect on the seed yield of any cultivar on either soil type, and was not associated with a positive or consistent effect on TSW. Herbage N concentration (%) was significantly ($P < 0.05$) and positively affected by fertiliser N application. The regression relationship was affected by cultivar, but not by soil type. Chlorophyll concentration (SPAD) was significantly ($P<0.05$) and positively correlated with herbage N concentration; the relationship was affected by cultivar but not by soil type. Seed yield was significantly related to spring herbage N concentration in only cv. Concord and Revielle on the Ultisol.

Additional index words: chlorophyll concentration, head numbers, seeds per head, SPAD, thousand seed weight, turf-type.

INTRODUCTION

Ryegrass seed crops are highly responsive to nitrogenous fertilisers, and applying nitrogen (N) in the correct amounts at the correct time is a key factor in the sustainability of ryegrass seed production (Cookson, Rowarth, Cornforth and Cameron, 1999). Maximum efficiency of N uptake is thought to occur during the late winter and early spring period and uptake is enhanced by prior application of N in autumn (Rowarth, Cookson, Williams and Cameron, 1999). Late application of N (e.g., after stem elongation) tends to result in an increase in vegetative, rather than reproductive, tillers (Rowarth, Cookson and Cameron, 1998a). However, these results have not been confirmed for Chile, where the balance between rainfall and evaporation is such that drainage continues in to October. This means that leaching of spring-applied N may result in a decrease in plant response.

In order to monitor plant N status, and hence manage N inputs, a reliable indicator of the amount of nitrogen needed to achieve a given seed yield within a season is required (Barraclough, 1997). Herbage N concentration (%) 14 days after spikelet initiation has been identified as a reliable indicator of relative seed yield in research trials in New Zealand (Rowarth and Archie, 1995). Chlorophyll concentration (SPAD) has also been shown to have potential as an instant, in-field indicator of plant N status (Wood, Reeves and Himelrick, 1993; Rowarth *et al.*, 1999).

This experiment was established to test the model identified for cv. Grasslands Nui (Rowarth, Boelt, Hampton, Marshall, Rolston, Sicard, Silberstein, Sedcole and Young, 1998) on Italian ryegrass (*Lolium multiflorum* Lam.) cv. Concord and Montblanc, and perennial ryegrass (*L. perenne* L.) cv. Revielle and Top Hat (a turf-type ryegrass), on two soil types (an Andisol and an Ultisol) near Temuco in Chile, 38.5° South and 73° West. The relationship between SPAD and herbage N concentration was also tested.

MATERIALS AND METHODS

Field trials were established on 16th May on an Andisol, Temuco Series and on 3rd June on an Ultisol, Nueva Imperial Series; details of the soils are given in Table 1a. At sowing, maintenance fertiliser, concordant with normal farmer practice for cereals, was applied (Table 1b).

Sowing rate was 7 kg ha^{-1} for cv. Concord, 10 kg ha^{-1} for cv. Montblanc and cv. Revielle, and 5 kg ha^{-1} for cv. Top Hat. Italian ryegrasses were sown in 170 mm rows and perennial ryegrasses in 340 mm rows. Cultivar plot size was 45.9 m^2 (3.06 x 15 m). Within these plots, N treatment was assigned randomly. There were no replicates.

Fifty kg ha^{-1} N was applied as 'Supernitro' (50% urea + 50% sodium nitrate) to all plots in mid July. Further N treatments

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Table 1. (a) Soil chemical data (0-200 mm).

	Andisol (Temuco)	Ultisol (Nueva Imperial)
[1]pH (H_2O)	6.1	5.8
[2]Organic matter (%)	14	9
[3]Nitrogen (mg kg^{-1})	43	29
[4]Phosphorus (mg kg^{-1})	11	22
[5]Potassium ($cmol_c$ kg^{-1})	156	344
[5]Sodium ($cmol_c$ kg^{-1})	0.29	0.44
[5]Calcium ($cmol_c$ kg^{-1})	8.62	7.14
[5]Magnesium ($cmol_c$ kg^{-1})	1.22	1.24
[6]Aluminium ($cmol_c$ kg^{-1})	0.01	0.03
Bases ($cmol_c$ kg^{-1})	10.53	9.70
Aluminium saturation (%)	0.10	0.31
[7]CICE ($cmol_c$ kg^{-1})	10.54	9.73
Boron (mg kg^{-1})	0.48	0.68
Sulphur (mg kg^{-1})	4	3

Extraction methods

1 pH	*Water: soil 2.5:1*
2 OM	*Walkley Black*
3 N	*KCl 2M*
4 P	*Olsen*
5 K, Ca, Na, Mg	*Ammonium acetate pH 7*
6 Exchangeable aluminium	*KCl 1M*
6 Extractable aluminium	*Ammonium acetate pH 4.8*
7 CICE	*Sum of bases plus aluminium (KCl)*

Table 1.(b) Maintenance fertiliser at sowing.

Fertiliser (kg ha^{-1})	Andisol (Temuco)	Ultisol (Nueva Imperial)
P_2O_5	147	120
K_2O	44	62
S	44	62
MgO	36	50
B	2	2
Zn	1	1

were applied to some plots at the end of September and/or the end of October (Table 2). Pest control was prophylactic. Irrigation was applied on 17th-19th November to the Andisol and on 24th October and 30th November to the Ultisol.

On 19th October chlorophyll concentration in herbage was measured using a Minolta 502 SPAD meter. Herbage was then sampled just above the leaf sheath and analysed for N using the Kjeldahl technique. Seed was harvested from 1.53 m^2 quadrats for the Italian ryegrasses and 1.36 m^2 quadrats for the perennial ryegrasses, between the 22nd and 29th December. After air drying, counting of reproductive heads, threshing and cleaning, seed yield and thousand seed weight (TSW) were recorded.

One way analysis of variance with repeated subsamples within each treatment was performed using Minitab 10.1. Pooled 5 % LSDs were calculated and are presented in the tables. Analyses of interactions were performed using Genstat. The analysis was based on there being plots within soil types. It is

Table 2. Effect of time and amount of applied nitrogen on seed yield of Italian ryegrass (cv. Concord and Montblanc) and perennial ryegrass (cv. Revielle and Top Hat) on two soil types.

Nitrogen strategy[1] (kg ha^{-1})	Andisol Concord	Andisol Montblanc	Andisol Revielle	Andisol Top Hat	Ultisol Concord	Ultisol Montblanc	Ultisol Revielle	Ultisol Top Hat
	Seed yield (kg ha^{-1})							
50-00-00	1723	2025	1059	1015	2139	2023	585	189
50-50-00	1668	2398	1442	1291	2095	2145	1536	644
50-50-100	1554	1969	1395	1102	2173	1862	1222	806
50-100-00	1593	1739	1483	1061	2533	2559	2687	986
50-100-50	1614	1734	1532	1156	2531	2099	1828	766
50-100-100	1489	1744	1286	1305	1959	1553	1830	669
Pooled LSD P<0.05 = 226								

1 Nitrogen was applied on 16th July to the Andisol and 24th July to the Ultisol. The second application was on the 29th September and the third on 28th October.

Table 3. Effect of time and amount of applied nitrogen on thousand seed weight of Italian ryegrass (cv. Concord and Montblanc) and perennial ryegrass (cv. Revielle and Top Hat) on two soil types.

Nitrogen strategy[1] (kg ha^{-1})	Andisol Concord	Andisol Montblanc	Andisol Revielle	Andisol Top Hat	Ultisol Concord	Ultisol Montblanc	Ultisol Revielle	Ultisol Top Hat
	Thousand seed weight (g)							
50-00-00	2.37	3.76	3.23	1.48	2.36	3.77	3.38	1.87
50-50-00	2.27	3.47	3.16	1.46	2.63	3.42	3.43	1.90
50-50-100	2.26	3.48	3.11	1.55	2.23	3.77	3.41	1.80
50-100-00	2.25	3.56	3.01	1.50	2.30	3.58	3.31	1.65
50-100-50	2.18	3.85	3.26	1.69	2.32	3.46	3.30	1.83
50-100-100	2.23	3.77	3.32	1.56	2.45	3.79	3.31	1.69
Pooled LSD P<0.05 = 0.21								

1 Nitrogen was applied on 16th July to the Andisol and 24th July to the Ultisol. The second application was on the 29th September and the third on 28th October.

Table 4. Effect of time and amount of applied nitrogen on seeds per head of Italian ryegrass (cv. Concord and Montblanc) and perennial ryegrass (cv. Revielle and Top Hat) on two soil types.

Nitrogen strategy[1] (kg ha^{-1})	Andisol Concord	Andisol Montblanc	Andisol Revielle	Andisol Top Hat	Ultisol Concord	Ultisol Montblanc	Ultisol Revielle	Ultisol Top Hat
	Seeds per head							
50-00-00	47	61	10	22	109	52	15	11
50-50-00	49	79	14	30	79	71	45	26
50-50-100	93	91	19	29	143	38	47	36
50-100-00	101	62	20	27	107	67	60	40
50-100-50	57	61	16	39	135	51	51	24
50-100-100	49	40	13	37	91	61	43	28
Pooled LSD P<0.05 = 27								

1 Nitrogen was applied on 16th July to the Andisol and 24th July to the Ultisol. The second application was on the 29th September and the third on 28th October.

acknowledged that there is potential for confounding of effects as there was no blocking for source of bias. However, the soil was even in type, and there were no obvious sources of variability such as shelter or uneven irrigation. Regression analysis was also performed using Minitab 10.1. The Cate-Nelson separation technique, which involves partitioning data into diagonally opposite quadrats (using cross hairs), was used to establish critical thresholds for responses; the more complete the separation, the better the indication of a threshold (Cate and Nelson, 1971).

RESULTS AND DISCUSSION

On the Andisol, 50 kg ha^{-1} N in September significantly increased seed yields in cv. Montblanc, Revielle and Top Hat; N had no significant effect on the seed yield of cv. Concord (Table 2). There were no further increases in seed yield from increasing N in September, or adding more N in October. In contrast, on the Ultisol, maximum seed yields in all cultivars were achieved with 100 kg ha^{-1} N in September (Table 2). This result is thought to reflect the greater organic matter and nitrogen content in the Andisol in comparison with the Ultisol (Table 1). Again, there were no increases in seed yield

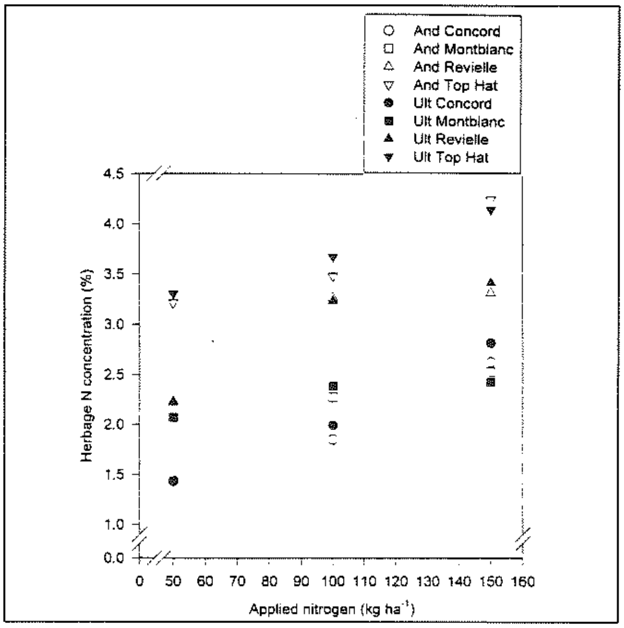

Figure 1. Effect of July and September nitrogen (kg ha^{-1}) on spring herbage nitrogen concentration (%) 21 days later.

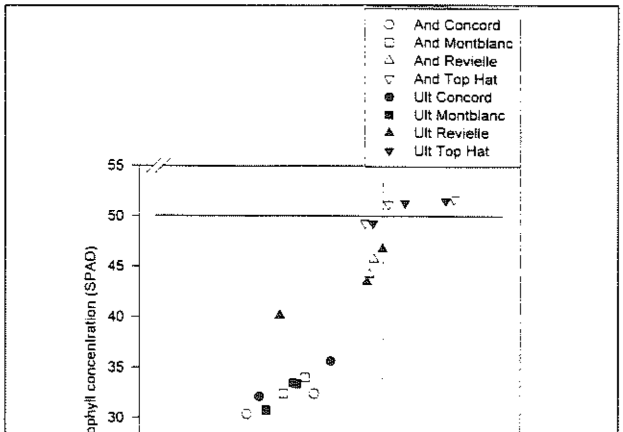

Figure 2. Relationship between spring herbage nitrogen concentration (%) and chlorophyll concentration (SPAD) in mid-October. Cate Nelson separation technique (Cate and Nelson, 1971) is used to show a critical threshold for SPAD sensitivity.

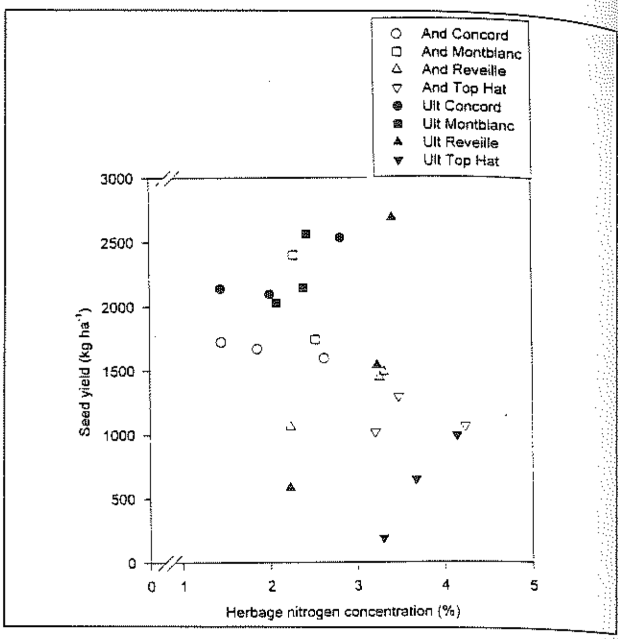

Figure 3. Relationship between spring herbage N concentration (%) 21 days after application of early spring nitrogen and ryegrass seed yield on two soil types.

in response to N applied in October. N applied after stem elongation has been shown to stimulate vegetative tillers, which do not contribute to seed yield at harvest (Meijer and Vreeke, 1988; Rowarth *et al.*, 1998a). Although dry matter of herbage was not measured at harvest, observation suggested that late N plots had more material, supporting results from research on timing of N and forage production in Chile (Teuber, Rosso and Winkler, 1988).

Nitrogen had no significant effect on TSW in cv. Concord and Top Hat on the Andisol, or in cv. Revielle on the Ultisol (Table 3). On the Ultisol, 50 kg ha^{-1} N in September significantly increased TSW in cv. Concord. In cv. Montblanc and Revielle on the Andisol, and cv. Montblanc and Top Hat on the Ultisol, TSW was significantly reduced by some N treatments. Nitrogen applied in late October did not have a consistent effect in either increasing TSW (which would have supported work by Nordestgaard (1986)) or decreasing TSW (suggesting that stimulation of secondary vegetative tillers caused competition for photoassimilate (Griffith, 1992)).

Nitrogen treatment had no significant effects on reproductive head numbers, suggesting that by the time differential treatments were applied, reproductive head numbers had been established. Average reproductive head numbers were 1290, 930, 3112 and 2767 m^{-2} for cv. Concord, Montblanc, Revielle and Top Hat, respectively, on the Andisol, and 920, 1076, 1175 and 1435 m^{-2} for the same cultivars on the Ultisol. Nitrogen did not affect seeds per head in cv. Revielle and Top Hat on the Andisol, or cv. Montblanc on the Ultisol (Table 4). In contrast, all September and October N treatments significantly increased seeds per head in cv. Revielle on the Ultisol. The other significant increases recorded depended on cutivar and soil type, and were in response to either 50 + 100 kg ha^{-1} N (September and October) or 100 kg ha^{-1} N (September only) (Table 4). Nitrogen treatments which gave the greatest seed yields were not necessarily those with the greatest number of seeds per head. Significant increases in seed yield were a result of small and variable changes in TSW, head numbers and seeds per head, supporting the frequently made observation that ryegrass has a great ability to compensate within its seed yield components.

Applied N had a significant and positive effect on spring herbage N concentration (%); the relationship was not affected by soil type (Fig. 1). At a given fertiliser N input, herbage N concentrations for the Italian ryegrasses were lower than for the perennial ryegrasses, supporting research in pot experiments (Rowarth, Archie and Baird, 1993; Rowarth and Archie, 1994; 1995). Regressions fitted to the individual

Table 5. Regression relationships for herbage N concentration (%) analysed 21 days after N application in September, and applied N (kg ha^{-1}). Data for soil type have been combined as there were no significant differences between relationships on the two soils.

Cultivar	Regression equation	R^2	P
Concord	Herbage N = 0.74 + 0.013 applied N[1]	0.96	0.000
Montblanc	Herbage N = 1.89 + 0.004 applied N	0.89	0.003
Revielle	Herbage N = 1.81 + 0.011 applied N	0.78	0.013
Top Hat	Herbage N = 2.74 + 0.009 applied N	0.93	0.001

1 Total N applied in July and September

Table 6. Regression relationships for chlorophyll concentration (SPAD) and herbage N concentration (%), analysed 21 days after nitrogen application in spring. Data for soil type have been combined as there were so significant differences between relationships on the two soils.

Cultivar	Regression equation	R^2	P
Concord	SPAD = 16.5 + 6.72 herbage N	0.83	0.007
Montblanc	SPAD = 15.3 + 7.51 herbage N	0.98	0.000
Revielle	SPAD = 29.5 + 4.73 herbage N	0.88	0.004
Top Hat	SPAD = 42.7 + 2.18 herbage N	0.64	0.035

Table 7. Regression relationships for seed yield and herbage N concentration (%) analysed in mid-October, 21 days after nitrogen application.

Soil type	Cultivar	Regression equation	R^2	P
Andisol	Concord	Seed yield = 1589 + 182 herbage N	0.00	0.57
Andisol	Montblanc	Seed yield = 2035 + 49 herbage N	0.00	0.95
Andisol	Revielle	Seed yield = -1383 + 968 herbage N	0.50	0.07
Andisol	Top Hat	Seed yield = -197 + 289 herbage N	0.00	0.54
Ultisol	Concord	Seed yield = 1875 - 109 herbage N	0.99	0.06
Ultisol	Montblanc	Seed yield = 3695 - 718 herbage N	0.00	0.67
Ultisol	Revielle	Seed yield = 204 + 383 herbage N	1.00	0.03
Ultisol	Top Hat	Seed yield = 1235 - 31 herbage N	0.00	0.93

responses combined for soil type showed that applied N accounted for 78-96% of the variability in herbage N concentration (%) (Table 5). The regression equations for herbage N concentration and applied N showed that applying 50 kg ha^{-1} would change the herbage N concentration by 0.65, 0.20, 0.55 and 0.45 % for cv. Concord, Montblanc, Revielle and Top Hat, respectively. For the perennial ryegrasses, the responses were similar to those reported for cv. Grasslands Nui in New Zealand, USA and Denmark (Rowarth *et al.*, 1998), and cv. Linn and Buccaneer in the USA (Young, Silberstein, Chastain and Rowarth, 1998).

Herbage N concentration (%) was directly related to chlorophyll concentration (SPAD) between 1.5 and 3.4 % N; all N concentrations above 3.4 % were associated with a SPAD of 50 (Fig. 2), indicating a critical threshold (Cate and Nelson 1971) for sensitivity of the chlorophyll meter. SPAD values for the Italian ryegrasses were lower (25-35) than those for the perennial ryegrasses (40-52), reflecting a lower N concentration (%). Regressions fitted to the individual responses combined for soil type showed that herbage N concentration accounted for 64 - 98 % of the variability in SPAD (Table 6).

Neither SPAD (data not presented) nor spring herbage N concentration were directly related to seed yield in most cultivars (Fig. 3). Regressions fitted to the individual responses showed that herbage N concentration accounted for a significant amount of the variability in seed yield only in cv. Concord and Revielle on the Ultisol (Table 7). Some of the lack of sensitivity was due to the limited number of samples in the analysis, but the fact that analyses were performed in mid-October, only two months from harvest, may also have been a factor. Chlorophyll concentration (SPAD) has been shown to be related to relative seed yield in perennial ryegrass (Rowarth *et al.*, 1999). Conditions of the research in lysimeters were, however, highly controlled and soil moisture was not limiting. When conditions are less controlled, and different cultivars, management systems and plant ages are involved, relationships are less easy to identify (Rowarth, Cookson and Cameron, 1998b).

CONCLUSIONS

In Chile for mid-May to early June sowings, there was no advantage in applying N in late October to ryegrass seed crops. On the Andisol, 50 kg ha^{-1} N in July, and 50 kg ha^{-1} N at the end of September were sufficient to achieve greater than 1600 kg ha^{-1} seed yield in Italian ryegrasses and greater than 1200 kg ha^{-1} in perennial ryegrass. In contrast, 50 kg ha^{-1} N in July and 100 kg ha^{-1} N at the end of September were required for greatest seed yields on the Ultisol (over 2500 kg ha^{-1} for the Italian ryegrass and perennial ryegrass cv. Revielle, but only 986 kg ha^{-1} in the turf-type perennial ryegrass). Although there was a close relationship between applied N, herbage N concentration and chlorophyll concentration (SPAD) in spring, these factors could not be used as an indicator of seed yield at harvest. The lack of relationship is thought to reflect the number of samples and timing of analysis. Further research is required in this new geographical area of ryegrass seed production to establish management systems which allow ryegrass seed to be produced sustainably.

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REFERENCES

1. Barraclough, P.B. 1997. N requirements of winter wheat and diagnosis of deficiency. *Aspects of Applied Biology* 50: 117-123.

2. Cate, R.B. and Nelson, L.A. 1971. A simple statistical procedure for partitioning soil test correlation data into two classes. *Soil Science Society of America Proceedings* 35: 658-660.

3. Cookson, W.R., Rowarth, J.S., Cornforth, I.S. and Cameron, K.C. 1999. Sustainability issues involved with nitrogen fertiliser use in perennial ryegrass (*Lolium perenne* L.) seed crops. *Journal of Applied Seed Production* 17: 67-76

4. Griffith, S.M. 1992. Changes in post-anthesis assimilates in stem and spike components of Italian ryegrass (*Lolium multiflorum* Lam.) I.Water soluble carbohydrates. *Annals of Botany* 69: 243-248.

5. Meijer, V.J.M. and Vreeke, S. 1988. Nitrogen fertilisation of grass seed crops as related to soil mineral nitrogen. *Netherlands Journal of Agricultural Science* 36: 375-385.

6. Nordestgaard, A. 1986. Investigations on the interaction between levels of nitrogen application in the autumn and time of nitrogen application in the spring to various grasses. *Journal of Applied Seed Production* 4: 16-25.

7. Rowarth, J.S. and Archie, W.J. 1994. The nutrient needs of small seed crops: a new concept in optimising seed yields. *Proceedings of the Agronomy Society of New Zealand* 24: 87-90.

8. Rowarth, J.S. and Archie, W.J. 1995. A diagnostic method for prediction of seed yield in perennial ryegrass. *Proceedings of the Third International Herbage Seed Conference*: 64-67.

9. Rowarth, J.S., Archie, W.J. and Baird, D.B. 1993. Nitrogen requirements for Italian ryegrass seed production. In: Plant nutrition from genetics to field practice, (ed. J.N. Barrow) 513-516, Kluwer Academic Publishers, Dordrecht.

10. Rowarth, J.S., Boelt, B., Hampton, J.G., Marshall, A.H., Rolston, M.P., Sicard, G., Silberstein, T.B., Sedcole, J.R. and Young, W.C.III. 1998. The relationship between applied nitrogen, nitrogen concentration in herbage and seed yield in perennial ryegrass (*Lolium perenne* L.). I. Cv. Grasslands Nui at five sites around the globe. *Journal of Applied Seed Production* 16: 105-114.

11. Rowarth, J.S., Cookson, W.R. and Cameron, K.C. 1998a. Optimising N application in ryegrass. *Foundation for Arable Research, Arable update herbage seed*, August, no 14. 2p.

12. Rowarth, J.S., Cookson, W.R. and Cameron, K.C. 1998b. Measuring nitrogen in ryegrass - relationship to seed yield. *Foundation for Arable Research, Arable update herbage seed*, August, no 13. 2p.

13. Rowarth, J.S., Cookson, W.R., Williams, P.H. and Cameron, K.C. 1999. Fate of nitrogen in perennial ryegrass (*Lolium perenne* L.) seed crops: can we alter destiny? *Proceedings of the Fourth International Herbage Seed Conference*: 91-95.

14. Teuber, N., Rosso, L. and Winkler, Ch. 1988. Niveles y frecuencia de aplicación de nitrógeno en ballica anual Sabalan. *Agricultura Técnica* 48(4): 370-374.

15. Wood, C.W., Reeves, D.W. and Himelrick, D.G. 1993. Relationships between chlorophyll meter readings and leaf chlorophyll concentration, N status, and crop yield: a review. *Proceedings of the Agronomy Society of New Zealand* 23: 1-9.

16. Young, W.C.III, Silberstein, T.B., Chastain T.G. and Rowarth. J.S. 1998. The relationship between applied nitrogen, nitrogen concentration in herbage and seed yield in perennial ryegrass (*Lolium perenne* L.). II. Cultivars in Oregon. *Journal of Applied Seed Production* 16: 115-124.