Selecting forage grasses for improved nitrate retention – a progress report

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Abstract
Significant variation for both root diameter and shoot dry weight was found among 20 commercially available hybrid and Italian ryegrass cultivars screened for root diameter. Nitrate interception by four selected cultivars was then compared with perennial ryegrass in a lysimeter trial. The shoot size of selected cultivars was significantly higher than perennial ryegrass. Drainage volumes from hybrid/Italian ryegrasses were half that of perennial ryegrass and contained significantly less nitrate. ^{15}N absorption was also significantly higher for thin (79-80%) and thick rooted (75-76%) hybrid/Italian cultivars than for the perennial ryegrass (63%). Root diameter of the different hybrid/Italian cultivars did not have a major impact on nitrogen interception and leaching. However the concept of a large, winter active grass with high nitrogen demand during periods of high leaching activity is supported by this study. Further selection for finer roots could increase the nitrogen interception ability of these plants without compromising shoot growth.
Keywords: leaching, nitrate, roots, ryegrass

Introduction
The deleterious effect on New Zealand surface and ground waters of nitrate lost from intensive farming has caused the sustainability of such systems to be questioned (Parliamentary Commissioner for the Environment 2004). Environment Waikato is proposing changes to its Regional Plan to limit nitrate leaching losses from farms in the lake's catchment (Environment Waikato 2007). If pastoral farming is to remain economic under these regulations, nitrogen (N) will have to be recycled more efficiently through the soil/plant/animal system, to allow production increases while not exceeding current nitrate losses.

Economic farm systems that leak less nitrate will probably involve combinations of several nitrate mitigation strategies (Ledgard *et al*. 2007). Our interest lies in development of forage cultivars that intercept more nitrate before it can be leached. High rates of nitrate interception are associated with active growth of large ryegrass plants with larger than average root systems (Crush *et al*. 2005, 2007). Heritability coefficients for root mass in ryegrass are adequate to allow breeding for this trait (Crush *et al*. 2006). The high-risk time for nitrate leaching in many New Zealand situations is late winter-early spring (Ledgard *et al*. 1998) when soils are saturated. This suggests that sowing hybrid or Italian ryegrasses with more active cool season growth than perennial ryegrass should help mitigate nitrate leaching. Thin roots intercept more nitrate per unit root weight than thicker roots (Dunbabin *et al*. 2003; Crush *et al*. 2005). Fine root systems impose a lower carbon cost per unit root surface area than thicker roots, potentially making more carbon available for shoot growth. Our current conceptual model of a nitrate-efficient ryegrass is a vigorous hybrid or Italian plant with a finely-divided root system. We screened commercially available cultivars to gauge the extent of variation in root diameter, and to see if any variation found had measurable effects on nitrate leaching.

Materials and Methods
Root traits of cultivars
Twenty cultivars of hybrid and Italian ryegrasses were selected for screening from cultivars commercially available in New Zealand in mid-2005. Ten replicates of each cultivar were grown in individual 15 cm diameter x 12 cm deep pots of sand, arranged in a randomised block design in a glasshouse at average day/night temperatures of 20.7/14.5°C. Plants received 125 ml of a complete nutrient solution (Hewitt 1966) three times per week and water on the remaining days.

After 70 days growth, the roots were washed out of the sand and samples collected by cutting a 1 cm wide strip across the root system 5 cm below the tiller base. These were preserved in 70% ethanol at 5°C. Shoots and the remaining roots were then separated, oven dried overnight at 70°C, and dry weights (DW) determined. The root samples were thoroughly mixed and digital images of subsamples were recorded and analysed for root diameter and length as described by Nichols *et al*. (2007). The preserved root samples were washed in water to remove ethanol, oven dried at 70°C for 6 hours, and weighed.

Nitrate interception experiment
From the results of the screening experiment, two cultivars with thinner roots (Tabu 0.241 mm, Marbella 0.245 mm) and two with thicker roots (Archie 0.272 mm, Cordura 0.282 mm) were chosen to test nitrate interception in a lysimeter trial that ran from early September to early December. Samson perennial ryegrass with AR1 endophyte was also included. The average

Table 1 Root and shoot parameters for 20 hybrid/Italian ryegrass cultivars. For letters in superscript, H = hybrid, I = Italian, T = tetraploid. Where known, endophyte status is indicated as: AR1, nil or wt (wildtype). Least significant differences (LSD) and (for back transformed values) least significant ratios (LSR) are tabulated.

	Root parameters				Shoot parameters	
Cultivar	Average diameter (mm)	SRL (cm/mg)	Estimated total root length (m)	Root dry wt (g)	Shoot dry wt (g)	Root:shoot
Archie [I, T]	0.272	33.7	875	2.52	3.90	0.66
Banquet [H, T, wt]	0.271	29.97	520	1.67	2.72	0.65
Concord [I]	0.268	33.57	1210	3.56	4.03	0.90
Conker [I]	0.267	34.35	1323	3.75	4.11	0.86
Cordura [I]	0.282	33.29	1139	3.33	4.93	0.71
Corvette [I]	0.255	34.29	1008	2.79	4.14	0.70
Crusader [I]	0.246	39.6	1468	3.37	4.43	0.79
Exalta [I]	0.275	35.85	1693	4.69	5.66	0.81
Flanker [I]	0.261	38.17	1066	2.86	3.57	0.71
Galaxy [H, T, AR1]	0.279	29.34	559	1.95	3.13	0.65
Geyser [I]	0.242	41.84	889	2.15	2.98	0.73
Greenstone [H, T, nil]	0.275	32.21	546	1.68	2.68	0.63
Marbella [I]	0.245	37.41	1230	3.38	4.38	0.75
Marsden [H, nil]	0.250	36.51	717	1.80	2.75	0.68
Maverick Gold [H]	0.259	33.16	962	2.87	3.99	0.73
Status [I]	0.255	36.54	997	2.73	4.21	0.69
Sterling [H, T, AR1]	0.265	29.74	447	1.54	2.75	0.59
Supreme [H]	0.273	31.29	971	2.88	3.69	0.77
Tabu [I]	0.241	39.46	1338	3.43	4.30	0.77
Warrior [I]	0.252	31.59	892	2.70	4.32	0.63
chi pr	0.002	0.268	<0.001	<0.001	<0.001	0.09
$LSD_{0.05}$	0.024	9.058			0.84	
$LSR_{0.05}$			1.59	1.41		1.29

root diameter of Samson with AR1 was 0.213 mm in a Taupo ash soil lysimeter experiment (A. J. Popay, pers. comm.).

Twenty five lysimeters (24 cm diameter x 50 cm deep) were set up in the glasshouse at average day/night temperatures of 19.8ºC/13.9ºC, using a randomised block design with five replicates of each cultivar. A 50 mm layer of gravel was placed in the bottom to aid drainage, and the lysimeters were filled with sand. Nine seedlings were transplanted into each lysimeter. Once the plants were established, all lysimeters received 2.2 mm of the complete nutrient solution containing the equivalent of 7.4 kg N/ha/application three times per week, and 2.2 mm water on the remaining days. Over the first 9 weeks after establishment, the plants were trimmed twice to promote tillering.

Sixty seven days after planting, N at 300 kg/ha N equivalent rate was applied to each lysimeter in the form of 1% enrichment $K^{15}NO_3$. For the next 26 days, known volumes of nutrients and water were applied as described above to provide 63 mm water input and the equivalent of 92 kg N/ha. For each lysimeter, the volume of leachate was recorded and a subsample was frozen and later analysed for nitrate by flow injection analysis to ISO standard 13395.

The plants were harvested 26 days after the ^{15}N application. Shoots were removed near the sand surface and the root/sand column cut into 0-10 cm, 10-20 cm and 20 cm+ depth sections. Washed roots and shoots were oven dried at 70°C for 24 hours and weighed. Root and shoot percent N in dry matter (DM) and ^{15}N Atom percent were analysed at the Lincoln University Analytical Services laboratory.

Statistical analyses

The data were analysed using the REML directive of Genstat 2005. Data for root DW, root/shoot DW ratio, and estimated root length were log transformed prior to analysis and, for these parameters, back-transformed means with least significant ratios (LSR) are presented in the tables.

Results

Root traits

There were significant differences among the cultivars for root diameter and average diameter ranged from 0.241 mm to 0.282 mm (Table 1). The two finest-rooted cultivars (Tabu, Geyser) had significantly thinner roots than the nine cultivars with the thickest roots. Overall, there was no significant difference in specific root length (SRL, cm/mg DW), despite ranging from 29.3 cm/mg (Galaxy) to 41.8 cm/mg (Geyser) (Table 1). Estimated

Table 2 Mean values for root and shoot parameters, drainage and nitrate uptake for two thin-rooted and two thick-rooted hybrid/Italian ryegrass cultivars and for perennial ryegrass cv. Samson. P values and LSDs for the perennial vs hybrid/Italian and thin vs thick rooted hybrid/Italian cultivars are given.

Parameter	Samson	Thin root	Thick root	P	LSD Samson v hybrid/Italian	LSD hybrid/Italian
Roots 0-10[1] (g)	30.8	37.7	33.3	ns	7.76	6.34
Roots 10-20[1] (g)	18.0	14.6	12.9	0.003	2.45	2.0
Roots 20+[1] (g)	18.2	19.1	17.6	ns	2.646	2.16
Total root wt (g)	67.0	71.4	63.8	ns	10.16	8.3
Shoot wt (g)	83.2	123.9	127.6	<0.001	8.32	6.8
Root:shoot	0.814	0.577	0.501	<0.001	0.1072	0.0874
Drainage (mm)	20.5	10.4	10.5	<0.001	2.8	2.3
Drainage (%)	32.55	16.47	16.61	<0.001	4.506	3.678
Root %N	1.019	0.800	0.887	0.002	0.0994	0.0812
Shoot %N	1.911	1.609	1.481	<0.001	0.1358	0.1108
Total plant N (g)	2.267	2.544	2.442	0.012	0.161	0.1314
^{15}N absorbed (%)	63.3	79.4	75.7	0.005	8.38	6.84
NO_3 leached (mg)	192	90	85	0.005	58.8	48

[1] Depth in cm at which roots were sampled

Table 3 Mean values for root and shoot parameters, drainage, shoot and root percent N and nitrate uptake for four hybrid/Italian ryegrass cultivars grouped by root diameter. P values for comparing cultivars within root type are given, with LSDs where there are significant differences.

Parameter	—— Thick roots ——		—— Thin roots ——		P	LSD
	Archie	Cordura	Marbella	Tabu		
Roots 0-10[1] (g)	41.2	25.4	34.0	41.4	0.005	8.96
Roots 10-20[1] (g)	14.2	11.6	13.4	15.8	0.071	
Roots 20+[1] (g)	17.2	18.0	18.6	19.6	0.709	
Total root wt (g)	72.6	55.0	66.0	76.8	0.01	11.74
Shoot wt (g)	127.8	127.4	120.0	127.8	0.294	
Root:shoot	0.57	0.43	0.55	0.60	0.088	
Drainage (mm)	9.9	11.0	11.5	9.2	0.333	
Drainage (%)	15.8	17.44	18.3	14.6	0.333	
Root %N	0.78	1.00	0.88	0.72	<0.001	0.11
Shoot %N	1.45	1.51	1.64	1.58	0.571	
Total plant N (g)	2.41	2.47	2.53	2.55	0.815	
^{15}N absorbed (%)	75.2	76.1	78.8	79.9	0.959	
NO_3 leached (mg)	69	102	78	102	0.493	

[1] Depth in cm at which roots were sampled

total root length ranged from 447 m (Sterling) to 1693 m (Exalta) and there were significant differences among the cultivars (Table 1). On an individual basis, only those cultivars at opposite extremes of the root length range were significantly different.

Root DW per plant ranged widely, from 1.544 g (Sterling) to 4.694 g (Exalta) (Table 1). Overall, variation between cultivars was significant, but as with other root traits, only cultivars at the extremes of the measured weight range were statistically significantly different. Shoot DW differed significantly between cultivars (Table 1), and ranged from 2.679 g for Greenstone to 5.659 g for Exalta. Exalta was significantly heavier than every other cultivar except Cordura. There were no statistically significant differences among the cultivars in root:shoot DW ratios, which ranged from 0.59 (Sterling) to 0.90 (Concord) (Table 1).

Nitrate interception experiment

Shoot weights of the perennial ryegrass (83.2 g/lysimeter) were significantly lower than those of the hybrid/Italian cultivars which all had similar shoot weights (mean value 125.7 g, Table 2). The only significant difference in root DW was in the 10-20 cm depth section (Table 2), where Samson had significantly more root mass than either of the thick or thin root selections. The thin selections (Tabu, Marbella) had heavier roots than the thick root selections (Archie, Cordura) but the difference was statistically significant only in the 10-20 cm zone. Root:shoot DW ratio in Samson (0.81) was significantly higher than in the hybrid/Italian selections (mean value 0.54).

The root and shoot N concentrations were significantly higher in the perennial ryegrass than in the hybrid/Italian ryegrasses (Table 2). Shoot percent N

values were generally low compared with pasture ryegrass samples because our samples contained a lot of tiller base material with low N. The shoot N concentrations were significantly higher in the thin rooted hybrid/Italian ryegrass cultivars than in the thicker rooted cultivars. Total plant N was lower in the perennial ryegrass than in the hybrid/Italian ryegrasses which did not differ significantly from each other (Table 2).

Drainage from the perennial ryegrass lysimeters was 20.5 mm compared to 10.4 mm from the hybrid/Italian ryegrasses. The leachate from perennial ryegrass accounted for 32.5% of the total water applied during the monitored irrigation phase, but only 16% of the applied water drained from the hybrid/Italian cultivars (Table 2). Perennial ryegrass lysimeters leached more nitrate and absorbed a significantly smaller proportion of the applied ^{15}N (63.3%) compared with the hybrid/Italian ryegrass lysimeters. Of the applied ^{15}N, 79% and 80% was retained by the thin root selections (Marbella and Tabu respectively) and 75% and 76% by the thick root selections (Archie and Cordura respectively).

There were few statistically significant differences in shoot and root measurements between the hybrid/Italian cultivars within each root type (Table 3). For the thick rooted hybrid/Italian cultivars, Cordura had a significantly lighter root system than Archie and a lower proportion of its root mass in the 0-10 cm depth zone. Shoot DW was very similar in all four cultivars, and root:shoot DW ratios did not differ significantly despite being much lower in Cordura. Root percent N was significantly higher in Cordura than in Archie and Marbella was higher than Tabu. Shoot percent N and total plant N did not vary among the cultivars. Total drainage volumes and nitrate content of the leachate did not differ between the cultivars within root type selections.

Discussion

Forage plant breeders have not so far attempted to combine root systems that are effective in nitrate interception with productive shoot growth. The survey of root traits in hybrid/Italian ryegrass cultivars was done to gauge the extent of "off-the-shelf" variation in root traits that might contribute to nitrate interception efficiency. Some variation between cultivars was recorded, but not surprisingly for a non-selected characteristic, there was substantial genotypic variation, and generally only cultivars at opposite ends of the range for any parameter were significantly different.

The cultivars also differed significantly in shoot weight, suggesting that it should be possible to select cultivars for high growth rates and nitrogen demand. Grasses for sustainable farming will have to balance root size for nutrient uptake against shoot size for animal production. Root diameter is known to affect nutrient uptake, with thin roots intercepting more nitrate per unit of DW than thick roots (Dunbabin *et al*. 2003; Crush *et al*. 2005). Nitrate interception by the hybrid/Italian ryegrasses selected for root diameter differed as anticipated although the magnitude of the difference was not great. This can be expected in non-selected plant material and further selection within the finer-rooted cultivars should lead to increased efficiency in nitrate interception. Rates of genetic gain for root traits are unknown for ryegrass, but were 2.4% per cycle in white clover (Caradus & Woodfield 1998) so several cycles of selection will be required to make an impact in grazed pasture.

Greater ^{15}N absorption and lower nitrate losses under hybrid/Italian ryegrasses probably reflect higher rates of water uptake and demand for nitrogen driven by larger shoot sizes. The lower water use efficiency (growth per unit water absorbed) of hybrid ryegrasses compared with perennials (Jensen *et al*. 2002) should also reduce drainage volumes and associated nitrate leaching. The lower shoot percent N in the hybrid ryegrasses compared to the perennial ryegrass will, if confirmed in the field, lead to better utilisation of dietary N and reduced N deposition in urine patches.

This study has shown that variation for root diameter does exist in commercially available hybrid and Italian ryegrasses in New Zealand, but this difference did not have a major impact on nitrate interception. However, the concept of a large, winter-active grass with greater N demand and uptake during times of high leaching activity does appear valid. Even without the potential benefits of thinner roots, basic differences in growth and water use efficiency appear sufficient to offer the possibility of reducing nitrate leaching. The performance of the selected cultivars will be tested in small plots in the Taupo catchment. Temperature regimes, growth rates, rainfall and drainage patterns, along with grazing, will affect water uptake and nitrate interception in the field. The results of field plot testing should provide guidelines to the type and extent of root system needed to reduce nitrate leaching.

ACKNOWLEDGEMENTS

Funding was provided through FRST contract C10X0315. We thank Phil Theobald and Dr John Waller of AgResearch for the leachate and statistical analyses respectively.

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