Establishment of ryegrass pastures containing a novel endophyte

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Abstract

An experiment was conducted at Dexcel, Hamilton from September 1999 to April 2001 to evaluate how pre-sowing pasture management and establishment method influenced the contamination of a newly sown AR1 endophyte-infected ryegrass dairy pasture with ryegrass infected with the wild endophyte (*Neotyphodium lolii*). Contamination level was estimated by counting the number of volunteer ryegrass plants between drill rows and by analysing bulk ryegrass samples for lolitrem B concentration. Hay, silage, grazed, grazed/topped and a turnip crop as pre-sowing managements generated large differences in viable ryegrass seed density (2555, 747, 348, 391 and 25 seeds/m^2, respectively) on the soil surface after natural reseeding in March 2000. Measurements on 20 November 2000 showed the spray/cultivation and double-spray/fallow establishment methods were effective in reducing contamination with volunteer ryegrass plants (8 and 34 plants/m^2, respectively), in contrast to drilling AR1 endophyte-infected ryegrass seed into hard-grazed existing pasture (581 plants/m^2). On 14 March 2001, lolitrem B levels were lower in the spray/cultivated and double-spray/fallow treatments compared to the hard-grazed treatment (0.3, 0.5, 1.1 µg/g, respectively). Information is also presented on sown plant density of AR1 endophyte-infected ryegrass.

Keywords: AR1 endophyte, cultivation, glyphosate, *Lolium perenne*, natural reseeding, *Neotyphodium lolii*

Introduction

Novel endophytes provide a way of avoiding animal health and production problems associated with ryegrass infected with the wild endophyte (*Neotyphodium lolii*), while maintaining positive effects on plant persistence. AR1 is an example of a novel endophyte, which does not produce the toxic alkaloids lolitrem B and ergovaline, but produces the insect deterrent peramine (Tapper & Latch 1999). AR1 was released in a number of commercial perennial ryegrass cultivars in autumn 2001. Grazing trials have shown that ryegrass containing AR1 endophyte can improve liveweight gains of sheep (Fletcher 1999) and milk production from dairy cows (Bluett *et al.* 2001) when compared with ryegrass containing the wild endophyte. For farmers to benefit from the potential advantages of novel endophytes, contamination from the wild endophyte must be minimised. This is best achieved by preventing natural reseeding before renovation, killing all existing vegetative ryegrass plants, depleting levels of buried ryegrass seed through cultivation or a fallow period, providing a seedbed which favours rapid establishment of the sown ryegrass, and by preventing seed dispersal in dung or hay (Hume 1999). The experiment reported in this paper was designed to assess the impact of pre-sowing management and establishment method on the contamination of a newly sown perennial ryegrass-based dairy pasture containing a novel endophyte.

Materials and methods

The experiment was conducted on 2 ha of endophyte-infected, perennial ryegrass-dominant dairy pasture at Dexcel (formally the Dairying Research Corporation), Hamilton, from September 1999 to June 2001. Soil types at the site were the Te Kowhai humic silt loam, Horotiu mottled silt loam and the Horotiu shallow silt loam (Singleton 1991). Fertiliser was applied on 15 September 1999, equivalent to 30 kg/ha of phosphorus, 3 kg/ha of sulphur, 26 kg/ha of calcium and 11 kg/ha of magnesium (granulated triple super® and calmag®: Summit Quinphos NZ Limited).

The experimental design was a split plot factorial, with five main plots (each 1275 m^2) replicated three times and each subdivided into three subplots (each 425 m^2), giving a total of 45 subplots.

Pre-sowing treatments (main plots)

Five pre-sowing main plot treatments were applied over the spring–summer of 1999/2000, to evaluate levels of contamination with endophyte-infected volunteer ryegrass over a range of natural reseeding levels. These were:

(a) *Hay making*
Plots were closed from grazing from 28 September 1999 to 12 January 2000. Hay was cut on 12 January, turned on 13 January, rowed and removed on 14 January. The hay yielded 9.2 tonnes/ha. Plots were then grazed at the same time as for (c).

(b) *Silage making*
Plots were closed from grazing from 28 September 1999 to 16 December 1999. Silage was cut on 16 December and removed on 17 December. The silage yielded 8.3 tonnes/ha. Plots were then grazed at the same time as for (c).

(c) *Rotational grazing*
Dairy cows grazed plots in accordance with normal farm management (mean grazing interval was 35 days from 28 September 1999 to 20 March 2000).

(d) *Rotational grazing followed by topping*
Dairy cows grazed the plots at the same time as for (c), followed by mechanical topping to decapitate ryegrass seed-heads.

(e) *Spring-sown turnips*
On 14 October 1999, plots were sprayed with Roundup® herbicide at 4 l/ha (1.44 kg glyphosate/ha) before grazing. Two weeks later, plots were ploughed and granular boron applied at 5 kg/ha before cultivation. Turnips (*cv*. Barabas) were sown at 3 kg/ha on 28 October 1999. The turnips yielded 8.2 tonnes/ha before being grazed by dairy cows from 26 January to 4 February 2000. After grazing the turnip plots were left fallow.

Establishment treatments (subplots)
In autumn 2000, the main plots were split into the three subplots to investigate the effects of establishment method on ryegrass contamination. These treatments were:

(i) *Spray/cultivation*
Subplots were sprayed with Roundup® herbicide at 4 l/ha on 20 April 2000, and were ploughed on 27 April and cultivated on 2 May 2000.

(ii) *Double-spray/fallow*
Subplots were sprayed with Roundup® herbicide at 4 l/ha on 9 February 2000 and were left fallow for 2 months. A second application of Roundup® herbicide (4 l/ha) was applied on 20 April 2000 after the emergence of volunteer ryegrass seedlings.

(iii) *Hard grazing*
Subplots were hard-grazed on 14 May 2000 to reduce competition from resident pasture (post-grazing residual was 900 kg DM/ha).

Sowing
All plots were direct-drilled with AR1 endophyte-infected perennial ryegrass (*cv*. Vedette) at 10 kg/ha, and white clover (*cv*. Grasslands Pitau) at 3 kg/ha on 21 May 2000. Following sowing, 10 kg/ha of Drisan insecticide (100 g/kg isazophos) was applied to control black beetle (*Heteronychus arator*). On 1 August MCPB herbicide was applied at 4 l/ha to control creeping buttercup (*Ranunculus repens*) and other broadleaf weeds.

Measurements

Pre-sowing (main plots)
Botanical composition
Bulked ground level herbage samples from each plot were collected monthly before grazing from September 1999 until March 2000, and were separated into ryegrass, other grasses, white clover, weeds and dead material.

Ryegrass seed-head density
Fortnightly counts of ryegrass seed-heads in five randomly placed 0.1 m^2 quadrats per plot were made from November 1999 until February 2000.

Viable ryegrass seed density
Viable ryegrass seed density in the soil was assessed on 27 September 1999 before pre-sowing treatments were imposed and again on 15 March 2000. Twenty 50-mm-diameter soil cores per plot to a depth of 50 mm were randomly collected. The upper 10 mm surface of each core was separated from the remainder and the two portions were analysed separately. The number of viable seeds in each portion was determined by counting ryegrass seedling germination in a controlled environment (Thompson & Grime 1979).

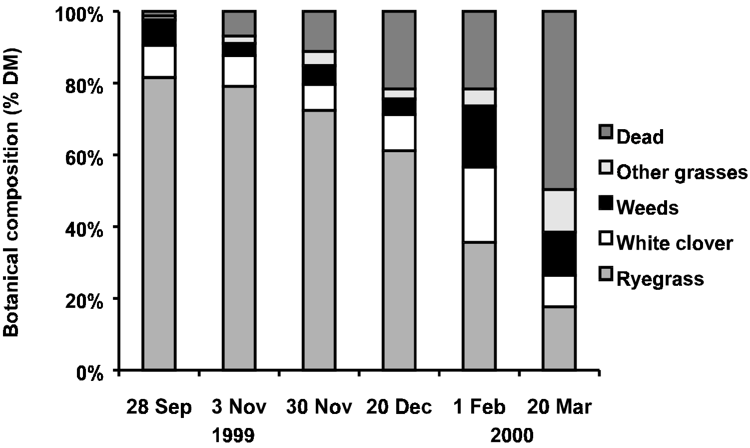
After sowing (subplots)
Botanical composition
Bulked ground level herbage samples from each subplot were collected monthly before grazing from November 2000 until March 2001, and were separated into ryegrass, other grasses, white clover, weeds and dead material.

Sown and volunteer ryegrass density
Three 104-mm-diameter rings were fixed in random positions on drill rows (sown ryegrass) and three

between drill rows (volunteer ryegrass) in each subplot in June 2000. All enclosed plants were tagged and their survival and tiller number were recorded monthly from June 2000 to April 2001 following the method of Burggraaf & Thom (2000). Volunteer ryegrass included old vegetative plants and new seedlings.

Lolitrem B concentration
Lolitrem B concentration was determined (Barker *et al.* 1993) on 9 January, 7 February and 14 March 2001 from the ryegrass component of bulked ground level herbage samples from each subplot and nearby resident pasture (control).

Figure 1 Botanical composition (% of DM) from September 1999 to March 2000, before sowing AR1 endophyte-infected ryegrass. Mean of hay, silage, grazed and grazed/topped pre-sowing treatments, excluding turnips (data omitted for silage on 20 December and hay on 1 February 2000, owing to plots just being harvested).



Statistical analysis
Analysis of variance was used to test for treatment differences using Genstat (version 4.1). A 5% P-value was used to determine significance. The data collected before sowing were analysed as a randomised complete block design and data collected after sowing as a split plot factorial. Data were analysed separately for each date. A square root transformation was used to analyse viable ryegrass seed density, sown and volunteer tiller and plant density, and a $\log_{10}$ transformation for lolitrem B concentration, because of heterogeneity of the variance. Treatment comparisons were made using Fishers protected LSD ($P<0.05$). As there were no significant interactions, only main effects of pre-sowing treatment and establishment method are presented.

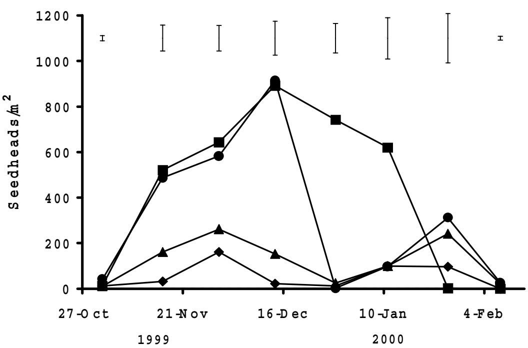
Results and discussion

Effect of pre-sowing treatment on pasture and ryegrass seed-head density

The botanical composition of the pasture before sowing AR1 endophyte-infected ryegrass consisted mainly of perennial ryegrass (*Lolium perenne*), hybrid ryegrass (*Lolium boucheanum* Syn. L. *hybridum*), white clover (*Trifolium repens*) and weeds (Figure 1). Weeds included yarrow (*Achillea millefolium*), creeping buttercup (*Ranunculus repens*), narrow-leaved plantain (*Plantago lanceolata*), hawksbeard (*Crepis capillaris*) and Californian thistle (*Cirsium arvense*). Pre-sowing management of the pasture treatments did not have any significant effects on the composition of the pasture from late spring through to autumn.

Pre-sowing treatments were designed to produce a range of natural reseeding levels of ryegrass, from maximum (hay) to minimal reseeding (turnips). The hay and silage plots produced over 900 ryegrass seed-heads/m^2 in mid-December (Figure 2). After this measurement, the silage was harvested on 16 December 1999 when seed-heads were green and immature. Further seed-heads developed over January and February owing to aftermath heading (Bluett *et al.* 1999; Burggraaf & Thom 2000). In contrast, the hay was harvested on 12 January 2000, when seed-heads were dry, mature and were shedding seed. Fewer seed-heads developed under rotational grazing, with topping

Figure 2 Ryegrass seed-head density (seed-heads/m^2) from November 1999 to February 2000 (hay -■-, silage -●-, grazed -▲- and grazed/topped -◆-) for pre-sowing treatments (no seedheads observed in turnips). Silage and hay was harvested on 16 December 1999 and 12 January 2000, respectively. Bars indicate average LSD, P< 0.05.



after grazing significantly reducing numbers from mid-November to mid-December 1999 (Figure 2). Hume & Lyons (1992) have reported similar seed-head numbers and treatment effects in the Manawatu.

Effect of pre-sowing treatment on natural reseeding

Pre-sowing treatment had a significant effect on the number of viable ryegrass seeds germinated from soil cores collected in March 2000 (Table 1). Hay making produced a large amount of viable seed (2674/m^2) compared to the other treatments. Because the silage was harvested before the seed had matured, viable seed numbers were similar to the grazed treatment. The turnip treatment had the lowest number of viable seeds present (33/m^2).

Most viable seed was on the soil surface or within the upper 10 mm (Table 1). However, some viable seed was located in the lower 40-mm horizon (5% in the hay treatment). Ryegrass seed deposited on the soil surface can be buried by a number of processes including livestock treading, water or wind movement into soil cracks (Hume 1999).

Soil cores collected in spring 1999 before the pre-sowing treatments were imposed contained a low number of viable ryegrass seeds (8/m^2). Ryegrass seed has low long-term survival because of a limited seed dormancy mechanism (Schafer & Chilcote 1970). However, in some soil environments, ryegrass seed and endophyte contained in the seed can survive up to 12 months burial in the soil (Hume 1999).

Table 1 Viable ryegrass seed density (seeds/m^2) from upper (10 mm) and lower (40 mm) horizons of soil cores collected on 15 March 2000. Raw and square root transformed data (in brackets) are presented.

Pre-sowing treatment	Upper 10 mm	Lower 40 mm
Hay	2555 (50)	119 (11)
Silage	747 (24)	76 (8)
Grazed	348 (17)	195 (14)
Grazed/topped	391 (19)	42 (6)
Turnips	25 (4)	8 (2)
SED	(6.7)	(2.0)
Significance	(**)	(**)

For all tables: NS = not significant, * = P<0.05, ** = P<0.01, *** = P<0.001

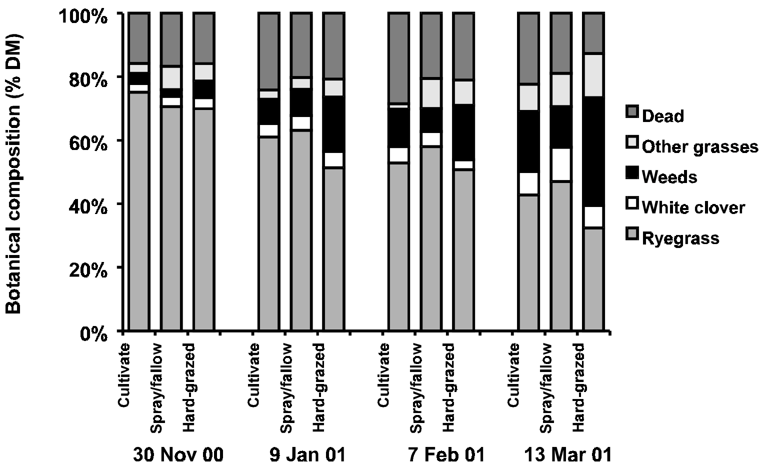
Table 2 Effect of establishment method on sown ryegrass plant density (plants/m^2) and tiller density (tillers/m^2) on 20 June 2000, 20 November 2000 and 17 April 2001 (mean of all pre-sowing treatments). Raw and square root transformed data (in brackets) are presented.

Establishment method	20 Jun 2000	20 Nov 2000	17 Apr 2001
Plant density			
Spray/cultivate	502 (20)	445 (18)	327 (15)
Double-spray/fallow	604 (23)	552 (22)	392 (17)
Hard-grazed	324 (14)	270 (12)	144 (7)
SED	(2.4)	(2.2)	(2.6)
Significance	(**)	(***)	(**)
Tiller density			
Spray/cultivate	502 (20)	6919 (74)	5114 (60)
Double-spray/fallow	604 (23)	7220 (79)	4997 (61)
Hard-grazed	324 (14)	1635 (27)	921 (18)
SED	(2.4)	(6.5)	(9.3)
Significance	(**)	(***)	(***)

Effect of establishment method on contamination

Method of establishment had a significant effect on the density of sown AR1 endophyte-infected perennial ryegrass (Table 2). Both plant and tiller numbers were lower in the hard-grazed treatment, because the resident pasture suppressed the growth of establishing seedlings and reduced their chance of survival (Thom *et al.* 1986). The hard-grazed plots also had a higher proportion of weeds than the spray/cultivated and double-spray/fallow plots (P<0.005) (Figure 3).

Figure 3 Effect of establishment method on botanical composition (% DM) after sowing AR1 endophyte-infected ryegrass (from November 2000 to March 2001).



The spray/cultivation and double-spray/fallow establishment methods used in this study resulted in a low level of contamination with volunteer ryegrass plants (Table 3). In contrast, the density of volunteer ryegrass plants or tillers (old and new) was significantly higher in the hard-grazed treatment. Lolitrem B concentration from ryegrass samples collected over summer and autumn were also higher from the hard-grazed treatment, compared with the spray/cultivated and double-spray/fallow treatments (Table 4). However, the hard-grazed treatment had lower lolitrem B concentrations than nearby resident pasture (3.1, 2.7 and 2.0 µg/g on 9 January, 7 February and 14 March, respectively) because of dilution with sown AR1 endophyte-infected ryegrass.

Lolitrem B concentrations in February and March 2001 were slightly higher in the double-spray/fallow treatment compared to the spray/cultivated treatment (Table 4), although this difference was not apparent in volunteer plant or tiller numbers (Table 3). Hume & Lyons (1992) reported higher contamination from areas that were sprayed rather than cultivated. There is a risk when using the double-spray/fallow method that volunteer ryegrass seedlings could germinate after the second herbicide application (Hume & Barker 1991). Young III & Youngberg (1996) concluded that minimum tillage systems were not sufficient to meet the cultivar purity standards for production of certified ryegrass seed. There is less risk of contamination using cultivation by ploughing because by inverting the sod, surface ryegrass seed is buried below the maximum depth of emergence, where *in situ* germination will result in the death of the seedlings (Schafer & Chilcote 1970). However, if a no-tillage system is used, a double rather than a single spray will significantly reduce contamination (Burggraaf & Thom 2000).

Table 3 Effect of establishment method on volunteer ryegrass plant density (plants/m^2) and tiller density (tillers/m^2) on 20 June 2000, 20 November 2000 and 17 April 2001 (mean of all pre-sowing treatments). Raw and square root transformed data (in brackets) are presented.

Establishment method	20 Jun 2000	20 Nov 2000	17 Apr 2001
Plant density			
Spray/cultivate	11 (1)	8 (1)	8 (1)
Double-spray/fallow	29 (2)	34 (3)	16 (1)
Hard-grazed	688 (21)	581 (20)	243 (12)
SED	(1.7)	(1.6)	(1.1)
Significance	(***)	(***)	(***)
Tiller density			
Spray/cultivate	11 (1)	515 (6)	173 (3)
Double-spray/fallow	99 (4)	1434 (16)	348 (6)
Hard-grazed	1873 (35)	4714 (59)	2310 (37)
SED	(2.8)	(6.3)	(5.4)
Significance	(***)	(***)	(***)

Table 4 Lolitrem B concentration (µg/g dry weight) from bulked ryegrass samples taken in summer/autumn of 2001 after establishment of AR1 endophyte-infected ryegrass using three methods. Raw and log^{10} transformed data (in brackets) are presented.

Establishment method	9 January	7 February	14 March
Spray/cultivate	0.1 (-1.0)	0.1 (-1.0)	0.3 (-0.6)
Double-spray/fallow	0.2 (-0.8)	0.3 (-0.7)	0.5 (-0.4)
Hard-grazed	1.0 (-0.1)	0.7 (-0.2)	1.1 (0.0)
SED	(0.11)	(0.12)	(0.09)
Significance	(***)	(***)	(***)

Effect of pre-sowing treatment on contamination

Sown ryegrass density was similar after all pre-sowing treatments, except on 20 November 2000 when tiller density was higher in turnips and on 17 April 2001 when plant density was higher in turnips and grazed treatments (Table 5). Pre-sowing management did not have a significant effect on the botanical composition of the new pasture at any date.

Because there were so few volunteer ryegrass plants in the spray/cultivated and double-spray/fallow treatments (Table 3), the effect of pre-sowing management was only analysed within the hard-grazed treatment (Table 6). The density of volunteer ryegrass plants was significantly lower in plots previously cropped with turnips (Table 6). However, because these volunteer plants did not have competition from resident plants, they were able to achieve a tiller density similar to the other pre-sowing treatments in November 2000 and April 2001 (Table 6). There was no significant effect of pre-sowing management on lolitrem B concentration from bulked ryegrass samples collected over summer and autumn. A more sensitive analysis of contamination by chemotyping individual tillers will be presented in a subsequent paper.

Table 5 Effect of pre-sowing treatment on sown ryegrass plant density (plants/m^2) and tiller density (tillers/m^2) on 20 June 2000, 20 November 2000 and 17 April (mean of establishment methods). Raw and square root transformed data (in brackets) are presented.

Pre-sowing treatment	20 Jun 2000	20 Nov 2000	17 Apr 2001
Plant density			
Hay	488 (20)	397 (17)	262 (13)
Silage	401 (17)	340 (14)	205 (10)
Grazed	623 (22)	571 (21)	397 (16)
Grazed/topped	375 (16)	349 (16)	235 (11)
Turnips	497 (20)	453 (19)	340 (15)
SED	(2.9)	(2.6)	(1.8)
Significance	(NS)	(NS)	(*)
Tiller density			
Hay	488 (20)	4827 (56)	3514 (45)
Silage	401 (17)	4177 (50)	2442 (34)
Grazed	623 (22)	5895 (65)	4408 (53)
Grazed/topped	375 (16)	3850 (52)	3514 (45)
Turnips	497 (20)	7543 (78)	4508 (53)
SED	(2.9)	(7.0)	(7.7)
Significance	(NS)	(*)	(NS)

Table 6 Effect of pre-sowing treatment on volunteer ryegrass plant density (plants/m^2) and tiller density (tillers/m^2) on 20 June 2000, 20 November 2000 and 17 April 2001 (mean of hard-grazed treatment only). Raw and square root transformed data (in brackets) are presented.

Pre-sowing treatment	20 Jun 2000	20 Nov 2000	17 Apr 2001
Plant density			
Hay	1805 (39)	1465 (36)	576 (22)
Silage	706 (25)	576 (23)	288 (15)
Grazed	641 (23)	563 (22)	170 (10)
Grazed/topped	248 (14)	261 (15)	144 (10)
Turnips	39 (4)	39 (4)	39 (4)
SED	(7.2)	(6.6)	(4.1)
Significance	(*)	(*)	(*)
Tiller density			
Hay	3152 (53)	6605 (77)	2812 (49)
Silage	2904 (49)	4787 (66)	2969 (44)
Grazed	2106 (41)	5075 (66)	1321 (28)
Grazed/topped	1072 (27)	3362 (51)	1635 (31)
Turnips	131 (6)	3741 (34)	2812 (30)
SED	(10.8)	(21.0)	(18.1)
Significance	(**)	(NS)	(NS)

Maintenance of the new pasture

After establishing a ryegrass pasture infected with a novel endophyte, ongoing management is required to prevent the reintroduction of wild-type endophyte from ryegrass seed in cow dung (Burggraaf & Thom 2000). Cows that have been grazing ryegrass seed-heads containing mature seed infected with wild endophyte should not be moved directly onto AR1-infected ryegrass pastures. A withholding period of at least 3 days is recommended to prevent seed being transferred in dung (Burggraaf unpublished data; Shelby 1991). In addition, hay cut from wild endophyte-infected ryegrass with mature seed-heads present should not be fed out on AR1-infected ryegrass pastures.

Conclusions

Pre-sowing management had a significant effect on the density of viable ryegrass seeds on the soil surface after natural reseeding. However, the spray/cultivation and double-spray/fallow establishment methods used in this study were effective in minimising contamination with volunteer ryegrass plants. In contrast, drilling AR1 endophyte-infected seed into hard-grazed pasture resulted in a poor establishment of sown ryegrass, and a high level of contamination because the presence of wild endophyte-infected ryegrass plants and viable ryegrass seed was not reduced.

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REFERENCES

Barker, D.J.; Davies, D.; Lane, G.A.; Latch, G.C.M.; Nott, H.M.; Tapper, B.A. 1993. Effect of water deficit on alkaloid concentrations in perennial ryegrass endophyte associations. pp. 67–71. *In*: Proceedings of the Second International Symposium on *Acremonium*/Grass Interactions. Eds. Hume, D.E.; Latch G.C.M; Easton, H.S. AgResearch Grasslands, Palmerston North, New Zealand.

Bluett, S.J.; Hodgson, J.; Kemp, P.D.; Barry, T.N. 1999. Survival, reproductive development and density of tillers in pure swards of Aries HD and Yatsyn 1 perennial ryegrass (*Lolium perenne*). *Proceedings of the New Zealand Grassland Association 61*: 197–201.

Bluett, S.J.; Thom, E.R.; Clark, D.A.; Tapper, B.A. 2001. Milk production and composition from cows grazing perennial ryegrass infected with a novel endophyte. pp.195–199. *In*: Proceedings of the 4th International Symposium on *Neotyphodium*/Grass Interactions. Eds. Volker H.P.; Dapprick, P.D. Paderborn University, Soest, Germany.

Burggraaf, V.T.; Thom, E.R. 2000. Contamination and persistence of endophyte-free ryegrass pastures established by spray-drilling and intensively grazed by dairy cows in the Waikato region of New Zealand. *New Zealand Journal of Agricultural Research 43*: 163–173.

Fletcher, L.R. 1999. "Non-toxic" endophytes in ryegrass and their effect on livestock health and production. Ryegrass endophyte: an essential New Zealand symbiosis. *Grassland Research and Practice Series 7*: 133–139.

Hume, D.E. 1999. Establishing and maintaining a toxin-free pasture: a review. Ryegrass endophyte: an essential New Zealand symbiosis. *Grassland Research and Practice Series 7*: 123–132.

Hume, D.E.; Barker, D.J. 1991. Natural reseeding of five grass species in summer dry hill country. *Proceedings of the New Zealand Grassland Association 53*: 97–104.

Hume, D.E.; Lyons, T.B. 1992. Establishment of new pastures free of ryegrass contamination. *Pro-*

ceedings of the New Zealand Grassland Association 54: 151–156.

Shelby, R.A. 1991. Survival of the tall fescue endophyte in the digestive tract of cattle and horses. *Plant Disease 75*: 776–778.

Schafer, D.E.; Chilcote, D.O. 1970. Factors influencing persistence and depletion of buried seed populations. *Crop Science 10*: 342–345.

Singleton, P.L. 1991. Soils of Ruakura – a window on the Waikato. Lower Hutt, New Zealand. DSIR Land Resources.

Tapper, B.A.; Latch, G.C.M. 1999. Selection against toxin production in endophyte-infected perennial ryegrass. Ryegrass endophyte: an essential New Zealand symbiosis. *Grassland Research and Practice Series 7*: 107–111.

Thom, E.R.; Sheath, G.W.; Bryant, A.M. 1986. Survival of overdrilled ryegrass in dairy pastures containing paspalum. *Proceedings of the New Zealand Grassland Association 47*: 141–148.

Thompson, K.; Grime, J.P. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology 67*: 893–921.

Young III, W.C.; Youngberg, H.W. 1996. Cropping systems for perennial ryegrass seed production: II. Minimum tillage systems for changing cultivars in certified seed production. *Agronomy Journal 88*: 78–82.

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