Survival of endophyte-free perennial ryegrass after autumn spray/ drilling in the Waikato

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Abstract

The persistence of an endophyte-free ryegrass (*Lolium perenne* L.) pasture established by autumn spray/drilling was studied over two years at the Dairying Research Corporation, Hamilton. Main plots were sprayed with glyphosate at 1.44 kg a.i./ ha (4 l/ha of Roundup G2) in mid March 1996 (S), or mid March and again in mid April (D). White clover (*Trifolium repens* L.) was removed from half the area of each main plot using herbicide and the remainder was drilled with white clover. All plots were direct drilled with endophyte-free peren-nial ryegrass in late April. Plots were rotationally grazed by dairy cows. Double spraying killed germinating volunteer ryegrass, *Poa* species and weeds, almost doubling the ryegrass content of D compared with S plots in the first winter/spring, and maintaining an advantage over the first year from drilling. Plants were larger in D than S over the first winter/spring, contributing to about a 10% improvement in their survival, reducing to about 5% by March 1998. Sown-plant densities were 200–250/m^2 by April 1998, similar to those found in high-endophyte pastures. Double spraying reduced the clover content of +Cl plots to similar levels as those in -Cl plots, lowering the potential herbage accumulation for D. Double spraying before drilling had a transitory effect on seasonal herbage accumulation, and no effect on overall herbage accumulation.

Keywords: dairy pastures, endophyte, persistence, plant competition, ryegrass, tillering, white clover

Introduction

Farmers currently have the option of sowing endophyte-infected (high-endophyte) or endophyte-free (low-endophyte) perennial ryegrass (*Lolium perenne* L.) seed.

Farmers have been advised to sow high-endophyte ryegrass in regions like the Waikato where Argentine stem weevil (*Listronotus bonariensis*) is a problem. The fungal endophyte *Neotyphodium lolii* produces peramine which promotes ryegrass persistence by deterring attack by Argentine stem weevil (Prestidge *et al.* 1982). The parasitic wasp *Microctonus hyperodae* may also reduce the influence of Argentine stem weevil, but does not replace the need for high-endophyte ryegrass (Thom 1997). However, grazing of high-endophyte ryegrass may cause ryegrass staggers, owing to the production of lolitrem B (Gallagher *et al.* 1981) or heat stress (Easton *et al.* 1996) in cattle.

Climate and competition for resources also affect ryegrass persistence. Tillering of seedlings is essential to their survival (Jewiss 1972), and a plant's ability to do this depends on availability of light and nutrients. Eliminating competition for these factors at the time of sowing aids establishment of ryegrass swards (Thom *et al.* 1986a). Double spraying with glyphosate before sowing in autumn greatly reduced volunteer plant density, increasing the survival of sown plants (van Vught & Thom 1997).

This paper reports on the first two years of a trial investigating the persistence of a low-endophyte ryegrass pasture rotationally grazed by dairy cows. It compares the effects of different rates of glyphosate herbicide and levels of white clover (*Trifolium repens* L.) on ryegrass growth and survival.

Methods

Trial design and treatments

At the Dairying Research Corporation, Hamilton, on a Te Kowhai clay loam soil, 20 × 16 m main plots were identified in March 1996 in a 0.25 ha paddock containing high-endophyte ryegrass (86% of tillers infected) and white clover. Plots were sprayed once (S) on 11 March 1996 with glyphosate at 1.44 kg a.i./ha (higher than the recommended rate of 1.08 kg a.i./ha; no penetrant was added), equivalent to 4 l/ha of Roundup G2. This was repeated on half of the plots on 12 April (double sprayed, D). Each plot was equally divided into two sub-plots; one was sprayed on 7 March with dicamba (0.6 kg a.i./ha or 3 l/ha of product) to kill white clover (-Cl) and the other drilled with Grasslands Kopu white clover (3 kg/ha) (+Cl) on 27 April. The trial area was direct-drilled with endophyte-free Yatsyn 1 ryegrass (10 kg/ha single pass) on 26 April 1996. Treatments were replicated 4 times in a randomised block design. The dicamba treatment was repeated in September and October 1997. Nitrogen fertiliser (40 kg N/ha) was applied to all plots on 10 December 1996.

Grazing management

The plots were rotationally grazed by dairy cows, stocked at 4 cows/ha, on 19 occasions, the first being 4 months after drilling. Average pre- and post-grazing herbage masses were 2.8 and 1.7 t DM/ha, respectively. Grazing intervals ranged from 16 to 39 days in spring–summer and from 35 to 63 days in autumn/winter.

Measurements

Sown and volunteer ryegrass density

Eight 10.4 cm diameter rings were fixed in random positions in May 1996 on drill rows (sown ryegrass, SR) and 8 between drill rows (volunteer ryegrass,VR) in each sub-plot. All enclosed plants were tagged with wire loops and their survival and tiller number monitored monthly from May 1996. VR germinating in autumn 1997 and 1998 was identified with different-coloured tags. In this paper the term "plant" refers to all plant material within the tag. Tags were periodically enlarged to allow for growth.

Botanical composition and herbage accumulation

Herbage clipped to ground level from each sub-plot at 2-monthly intervals was dissected into perennial ryegrass, *Poa* species, *Paspalum* species, other grass species, white clover, weeds and dead material of all species.

Forty readings with a calibrated pasture probe were made on each sub-plot before and after grazing to estimate herbage dry matter accumulation.

Endophyte screening

In August 1996, March 1997 and March 1998, tillers of 20 ryegrass plants per sub-plot were assessed for endophyte presence (Latch *et al*. 1984).

Results

Climate and insect effects

In the establishment autumn (March to May 1996) total rainfall was 143 mm (10-year average 91 mm). Over winter 1997, there was 239 mm of rain, 122 mm below the 10-year average. Frost incidence was also 17 less than the 10-year average of 55. Summer 1997 had 18 days when the screen maximum air temperature was above 25°C, compared with 37 days over the following summer. Both summers were dry, with 72 and 82% of the 10-year average rainfall (239 mm). There was only 37 mm of rain on 16 days from 27 December 1997 to 21 February 1998; the dry weather was accompanied by screen maximum air temperatures above 25°C on 26 days of this 56-day period. Autumn 1998 was dry, with only 74% of the 10-year average rainfall for March and April.

The parasitoid of Argentine stem weevil was released near the trial site in the winter of 1991. By winter 1995 it had spread throughout No. 5 Dairy and parasitism of stem weevil adults was 60–90% in most paddocks. Stem weevil populations were low on the dairy throughout the trial, and no larval or adult damage was noted on ryegrass tillers on the trial area (S.L. Marshall pers. comm.).

Endophyte

Endophyte-infected ryegrass was detected in all plots; levels in those double sprayed were at least 2–3 times lower than in those sprayed once (Table 1). Clover content had no effect on endophyte levels.

Table 1 Effect of single and double applications of glyphosate on endophyte content (% of plants infected), 4, 11, and 23 months after sowing.

Spray treatment	August 1996	March 1997	March 1998
Single	43	18	28
Double	8	7	9
SED	3.6	3.1	6.4
Significance	**	*	†

† = P<0.10; * = P<0.05; ** = P<0.01

Sown plant number

D plots had similar sown plant numbers (about 470 plants/m^2) from the first measurement (May 1996) until 6 months after sowing (October 1996) (Figure 1). S plots had slightly less plants (408/m^2) in May 1996 than did D, and numbers peaked in July 1996 (440 plants/m^2) before a sharp decline of 11% by September 1996, reaching significance (P<0.1) in October 1996 and January 1997. Plant numbers were slightly higher in -Cl than +Cl plots at their peak, with 471 and 445 plants/m^2, respectively.

More plants survived the first winter in D than S plots, but differences were not significant. For the remainder of the trial reductions in the number of surviving plants were similar across all treatments, and were greatly influenced by season and "pulling" during grazings. Losses were most rapid over summer/autumn 1997 (February to June), when a total of 84 plants/m^2 was lost; 34% of these losses were directly related to pulling of plants or clumps of plants. By June 1997, numbers had stabilised at 344 and 261 plants/m^2 for D and S, respectively, with intermediate ryegrass survival in -Cl and +Cl treatments.

Ryegrass numbers further declined over summer/autumn 1997/98 (December to April). Monthly losses from pulling were highest in January 1998 during drought conditions, when 4% (12 plants/m^2) of the remaining plants were removed. Over the trial period 28% of total

Figure 1 Effect of clover (Cl) and single (S) and double (D) applications of glyphosate on sown ryegrass plant density. For differences between S and D treatments † = P<0.1, * = P<0.05, ** = P<0.01.

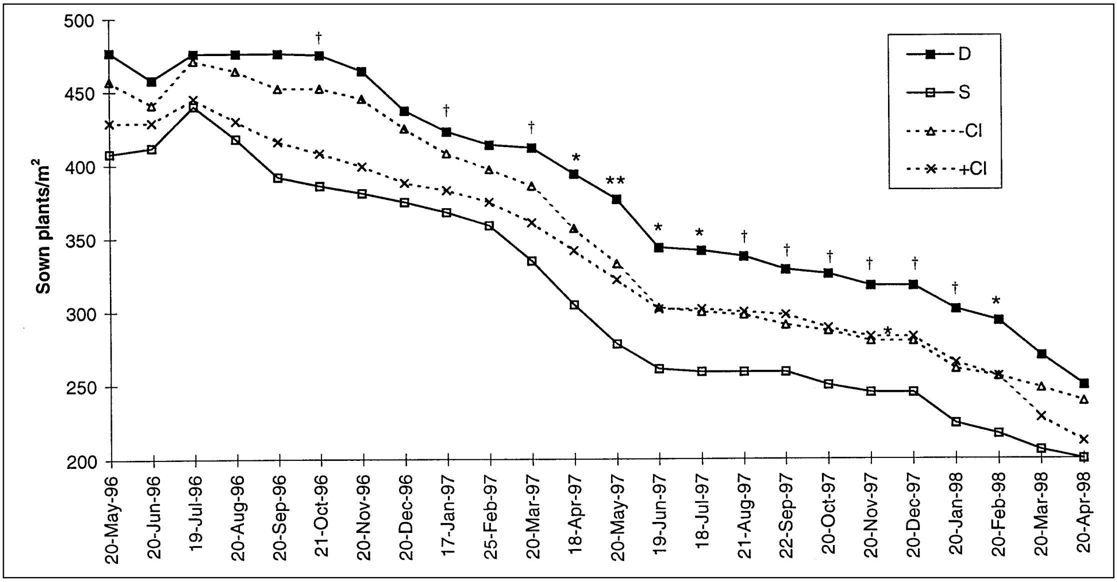
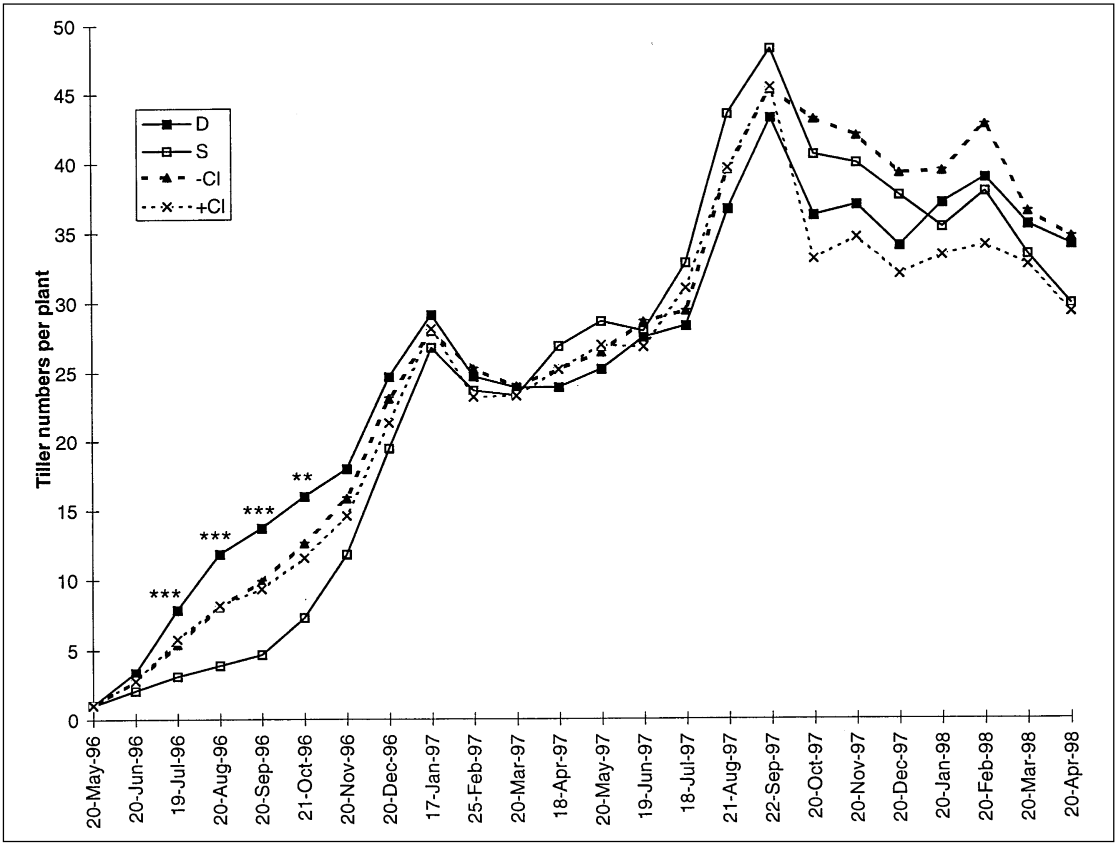


Figure 2 Effect of clover (Cl) and single (S) and double (D) applications of glyphosate on tiller numbers for sown ryegrass plants. For differences between S and D treatments ** = P<0.01, *** = P<0.001.



losses were owing to pulling. Differences between S and D plant numbers were often not significant at $P<0.05$, but in October 1996, January and March 1997, and August 1997 to January 1998 were significant at $P<0.1$. Sown plant numbers in April 1998 were 250 and 200 plants/m^2 for D and S, respectively. No interactions between spray and clover treatments were significant.

Plant size and tiller density

Sown ryegrass in D plots produced more tillers than S over the first winter and spring (July to October 1996) ($P<0.001$), and remained larger until January 1997, when all treatments reached about 28 tillers/plant (Figure 2). Ryegrass plant size peaked in September 1997 at 46 tillers/plant, and remained high in plots resprayed with dicamba in September/October 1997, relative to unsprayed (+Cl) plots. From January to December 1997 and January to March 1998, interactions between ryegrass plant size and clover treatment were significant at $P<0.05$ or $P<0.10$. During 1997, plants in S +Cl plots were often significantly larger than those in D +Cl with the opposite tendency in -Cl; however, in 1998, larger plants in D -Cl contributed most to significant interactions.

All treatments had a maximum sown ryegrass tiller density of about 13 000 tillers/m^2 in September 1997, declining to an average level of 7300 tillers/m^2 by April 1998.

Volunteer ryegrass plant number and size

A small ingress of volunteer ryegrass plants was recorded during the establishment autumn (1996) and was added to each autumn as further seed germination occurred. Numbers in S were 2–3 times those in D plots and by April 1998 had reached 57 and 22 plants/m^2, respectively.

Botanical composition

Ryegrass content tended to be greater in D plots, except in December 1997. The greatest separation occurred in October 1996 with 72% of DM in D compared with 44% in S ($P<0.05$) (Table 2). The ryegrass content of D plots increased from 50% in August 1996 to 90% by June 1997, declining to 50% by February 1998, before rising again to 69% in March 1998.

Significant interactions between the glyphosate spray treatment and clover treatment occurred in October and December 1996 and February and June 1997 whereby the white clover content of all D plots was similar to that of the single-sprayed -Cl (averaging about 4.5% of DM), until respraying with dicamba. Single-sprayed +Cl plots averaged 8 times more clover than all other treatments in October 1996 ($P<0.05$) and February 1997 ($P<0.01$). The average white clover content of +Cl plots was highest in February 1997 at 15.7% compared with 8.1% for -Cl.

Poa species were higher in S than D plots in August (54 vs 21%), reaching significance in October (38 vs 21%, $P<0.01$) 1996; thereafter the *Poa* content of all plots was low (0–3% of DM).

Table 2 Effect of single and double applications of glyphosate on ryegrass content (% of DM).

Date	Single spray	Double spray	SED
26 Aug 96	25	50	14.2 ^{NS}
17 Oct 96	44	72	6.6 *
10 Dec 96	63	76	7.1 ^{NS}
17 Feb 97	59	70	3.9 †
24 Apr 97	74	85	2.6 *
9 June 97	86	90	2.1 ^{NS}
13 Aug 97	82	92	4.5 ^{NS}
8 Oct 97	80	82	3.4 ^{NS}
5 Dec 97	79	77	2.8 ^{NS}
23 Feb 98	45	50	4.7 ^{NS}
31 Mar 98	61	69	6.7 ^{NS}

NS = not significant, † = $P<0.10$, * = $P<0.05$

In February 1998 there was a high proportion of other volunteer grass species, especially in plots where no clover was sown (24% of DM), and in S (21% of DM) rather than D (13% of DM) plots. These included summer grass (*Digitaria sanguinalis*), witchgrass (*Panicum dichotomiflorum*), mercer grass (*Paspalum distichum*), *Paspalum dilatatum* and prairie grass (*Bromus willdenowii*). This trend continued into March, with 17% in -Cl compared with 8% in +Cl ($P<0.05$), the predominant species being prairie grass.

There was little difference in the weed content of the plots, which ranged from 1–12% of DM, while dead matter contents of all plots were highest in February 1998 (28% of DM).

Herbage accumulation

The first winter measurement, 4½ months after the second spraying of glyphosate, showed S plots had accumulated 0.8 t DM/ha more than D (Table 3). However, over spring the trend reversed with the growth in D exceeding S by a similar amount. Herbage accumulation was similar for each treatment over summer and autumn, and consequently total herbage accumulation for the first year was similar. Treatment herbage accumulations over the following winter and spring were also similar; however, growth was greater in S than D plots for summer 1998, resulting in a higher total yield in the former for the second year.

Table 3 Effect of single and double applications of glyphosate before drilling on total herbage accumulation (t DM/ha).

Period	Single spray	Double spray	SED
Year 1 (1996/97)			
Winter[a]	2.8	2.0	0.11 **
Spring (2 Sep–16 Dec)	3.7	4.4	0.32 NS
Summer (18 Dec–13 Feb)	3.6	3.7	0.10 NS
Autumn (17 Feb–9 June)	4.2	4.3	0.18 NS
Total (409 days)	14.3	14.4	0.29 NS
Year 2 (1997/98)			
Winter (11 June–8 Oct)	3.5	3.5	0.08 NS
Spring (13 Oct–5 Dec)	2.0	2.1	0.07 NS
Summer (10 Dec–23 Feb)	2.6	2.1	0.10 *
Autumn (27 Feb–31 Mar)	0.9	0.9	0.09 NS
Total (294 days)	9.0	8.6	0.20 *

[a] Winter data are represented by the first pre-grazing measurement (30 August 1996), following drilling on 26 April 1996.
NS = Not significant; * = P<0.05; ** = P<0.01

Discussion

Most farmers renovate dairy pastures by undersowing in autumn. Thom *et al.* (1993) has shown distinct advantages to using a herbicide before drilling (spray/drilling) in promoting growth and persistence of the new grass. However, the current trial also had the objective of reducing contamination of endophyte-free ryegrass with endophyte-infected volunteer ryegrass (van Vught & Thom 1997), and so a second herbicide spray was used one month after the first. This provided a further opportunity to reduce competition between sown ryegrass and volunteer grasses and weeds germinating after the first spray.

The second spray killed germinating volunteer ryegrass, *Poa* species and weeds. This was reflected strongly in the near doubling of the ryegrass content of D compared with S plots in the first winter/spring after spraying (Table 2) and larger sown ryegrass plants during the same period (Figure 2), contributing to about a 10% improvement in survival of plants in D compared with S plots. This difference was maintained until the end of the second summer (March 1998), when it reduced to about 5%.

Even though survival of sown ryegrass was consistently better in D than S plots it did not improve total herbage accumulation (Table 3); however, the contribution of the desired species, ryegrass, was consistently greatest in D plots over the first year from drilling. The double spraying operation takes longer than spraying once before drilling, and so may delay the first grazing of the new pasture until spring; spraying first at the beginning of March would minimise this possibility. However, double spraying provided a practical option for maintaining a low-endophyte pasture (<10% of plants infected (van Vught & Thom 1997)) for at least 2 years.

Plant density and size

Sown plant densities declined on average from 458 to 225 plants/m^2 over the two years of the trial. Despite the low endophyte content of the plots, densities were still within the range of 200–600 plants/m^2 described by Thom *et al.* (1993) for established high-endophyte ryegrass pastures in the Waikato. No damage by Argentine stem weevil was observed and weevil populations on the trial were low, possibly owing to the activity of the parasitoid of the weevil (P.J. Addison pers. comm.). However, some tiller and plant losses over summer may still have been owing to this pest. Despite this possibility, tiller densities after 2 years were 1.5 to 3 times higher than those previously reported in the Waikato (L'Huillier 1987; Thom 1991).

Establishment numbers were better in D than S, owing to less competition with volunteer ryegrass, *Poa* species and weeds, allowing increased tillering and promoting better plant survival (Charles 1961). Loss of grass plants as individuals increase in size and ability to survive, has often been observed during the first year after sowing (Langer *et al.* 1964; Hoen 1968).

The pattern of high losses of newly sown endophyte-free ryegrass plants during the first summer/autumn mimics that for high endophyte ryegrass (Thom *et al.* 1986b; Thom *et al.* 1993). However, pulling made a greater contribution to losses than was reported by Thom *et al.* (1986a). Exceptionally dry and warm weather in summer/autumn 1998 continued the pattern of losses to levels of about 200–250 plants/m^2, which were similar to stable plant numbers in other dairy pastures established by spray/drilling (Thom *et al.* 1993).

Since volunteer perennial ryegrass seedlings may contain viable endophyte (van Vught & Thom 1997), their slow increase in numbers with time may gradually increase the endophyte content of the plots.

Herbage accumulation

The effect of double spraying on seasonal herbage production was restricted to the establishment winter and spring of 1996, and summer of 1998. Suppression of white clover growth by high rates of glyphosate herbicide is well recognised (e.g., Thom *et al.* 1993). This effect was probably partly responsible for the larger ryegrass plants in S +Cl plots, where the higher white clover contents improved the nitrogen supply (Sears *et al.* 1965). Double spraying initially produced less herbage than S as volunteer ryegrass, *Poa* and weeds were killed by the second spraying, and the sown ryegrass had not yet had a chance to establish. This loss was compensated for in spring 1996 as the larger numbers of ryegrass plants in D plots (Figure 1) began increasing in size (Figure 2), thereby increasing the ryegrass content of the double sprayed plots over those sprayed once.

As shown by Thom *et al.* (1993), high spring ryegrass content does not necessarily translate into higher summer yield (Table 3), especially since conditions were warm and dry. Summer herbage accumulations were further reduced in S and D plots in 1997/98 compared with 1996/97, as dry, warm weather was more intense with maximum daily screen temperatures above the optimum for ryegrass of 25°C (Mitchell 1956), favouring the growth of summer growing volunteers like *Digitaria sanguinalis*. These species contributed more to the yield of S than to D plots in summer 1997/98, and to higher annual yields in S.

Conclusions

Autumn spray/drilling was successfully used to establish a low-endophyte ryegrass pasture. The ryegrass content of double-sprayed areas was higher during the first year after drilling than those sprayed once. However, this did not produce large increases in the ryegrass plant population or seasonal and total annual pasture yield. Double spraying maintained a low level (<10% of plants infected) of contamination with endophyte-infected volunteer ryegrass.

A high-yielding pasture, dominated by endophyte-free ryegrass, was successfully maintained for 2 years under intensive grazing by dairy cows, where the parasitoid of stem weevil was present and when stem weevil populations were low.

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REFERENCES

Charles, A.H. 1961. Differential survival of cultivars of *Lolium*, *Dactylis* and *Phleum*. *Journal of the British Grassland Society 23*: 20–25.

Easton, H.S.; Lane, G.A.; Tapper, B.A.; Keogh, R.R.; Cooper, B.M.; Blackwell, M.; Anderson, M.; Fletcher, L.R. 1996. Ryegrass endophyte-related heat stress in cattle. *Proceedings of the New Zealand Grassland Association 57*: 37–41.

Gallagher, R.T.; White, E.P.; Mortimer, P.H. 1981. Ryegrass staggers; isolation of potent neurotoxins lolitrem A and lolitrem B from staggers producing pastures. *New Zealand veterinary journal 29*: 189–190.

Hoen, K. 1968. The effect of plant size and development stage on summer survival of some perennial grasses. *Australian journal of experimental agriculture and animal husbandry 8*: 190–196.

Jewiss, O.R. 1972. Tillering in grasses – its significance and control. *Journal of the British Grassland Society 27*: 65–82.

Langer, R.H.M.; Ryle, S.M.; Jewiss, O.R. 1964. The changing plant and tiller populations of timothy and meadow-fescue swards. *Journal of applied ecology 1*: 197–208.

Latch, G.C.M.; Christensen, M.J.; Samuels, G.J. 1984. Five endophytes of *Lolium* and *Festuca* in New Zealand. *Mycotaxon 20*: 525–550.

L' Huillier, P.J. 1987. Tiller appearance and death of *Lolium perenne* in mixed swards grazed by dairy cattle at two stocking rates. *New Zealand journal of agricultural research 30*: 15–22.

Mitchell, K.J. 1956. Growth of pasture species under controlled environment. I. Growth at various levels of constant temperature. *New Zealand journal of science and technology 38A*: 203–216.

Prestidge, R.A.; Pottinger, R.P.; Barker, G.M. 1982. An association of *Lolium* endophyte with ryegrass resistance to Argentine stem weevil. *Proceedings of the 35th New Zealand Weed and Pest Control Conference*: 199–122.

Sears, P.D.; Goodall, V.C.; Jackman, R.H.; Robinson, G.S. 1965. Pasture growth and soil fertility VIII. The influence of grasses, white clover, fertilisers, and the return of herbage clippings on pasture production of an impoverished soil. *New Zealand journal of agricultural research 8*: 270–283.

Thom, E.R.; Sheath, G.W.; Bryant, A.M.; Cox, N.R. 1986a. Renovation of pastures containing paspalum. 2. Effects of nitrogen fertiliser on the growth and persistence of overdrilled ryegrass. *New Zealand journal of agricultural research 29*: 587–598.

Thom, E.R.; Sheath, G.W.; Bryant, A.M.; Cox, N.R. 1986b. Renovation of pastures containing paspalm. 3. Effect of defoliation management and irrigation on ryegrass growth and persistence. *New Zealand journal of agricultural research 29*: 599–611.

Thom, E.R.; Wildermoth, D.D.; Taylor, M.J. 1993. Growth and persistence of perennial ryegrass and white clover direct-drilled into a paspalum-dominant dairy pasture treated with glyphosate. *New Zealand journal of agricultural research 36*: 197–207.

Thom, E.R. 1991. Effect of early spring grazing frequency on the reproductive growth and develop-ment of a perennial ryegrass tiller population. *New Zealand journal of agricultural research 34*: 383–389.

Thom, E.R. 1997. Endophytes in ryegrass; their importance on dairy farms. *Dairyfarming annual 49*: 150–156.

van Vught, V.T.; Thom, E.R. 1997. Ryegrass contamination of endophyte-free dairy pastures after spray/drilling in autumn. *Proceedings of the New Zealand Grassland Association 59*: 233–237.

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