Ryegrass production in Wairarapa, New Zealand: is biological control of Argentine stem weevil important?

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Abstract

To investigate the interaction between Argentine stem weevil (*Listronotus bonariensis*), its parasitoid *Microctonus hyperodae,* fungal endophyte (*Neotyphodium lolii*) and its grass host, four endophyte-ryegrass (*Lolium perenne*) treatments were established on four farms in Wairarapa in autumn 2003. A diploid ryegrass contained either wild-type or AR1 endophyte or was endophyte-free, while a tetraploid ryegrass contained AR1 endophyte. Wild-type, AR1 and endophyte-free ryegrasses average 52%, 91% and 2% endophyte-infected tillers for the 3 years of measurement. Moderate increases over time in the proportion of wild-type infected tillers indicated selection pressures favoured endophyte-infected tillers. Endophyte-free tiller populations were 11% lower than AR1 or wild-type but not significantly so. *L. bonariensis* populations were highest in endophyte-free and wild-type pastures and 80% lower in AR1 pastures. Mean rates of parasitism in *L. bonariensis* were lower in spring (9%) than autumn (35%), tended to be higher in wild-type than in AR1 diploid ryegrass, and only had a weak relationship with percent endophyte-infection.

Keywords: endophyte, *Neotyphodium lolii*, *Lolium perenne*, dry matter yields, plant persistence, *Listronotus bonariensis*, *Microctonus hyperodae*

Introduction

The parasitoid *Microctonus hyperodae* (Hymenoptera: Braconidae) was first released in New Zealand in 1991 in what was a classical biological control programme targeting Argentine stem weevil (*Listronotus bonariensis*) (Kuschel) (Coleoptera: Curculionidae). *L. bonariensis* is a well known pest of ryegrass (*Lolium* spp.) in New Zealand (e.g. Goldson 1982), adding a significant cost to the pastoral industry (Prestidge *et al.* 1991). The parasitoid attacks adult *L. bonariensis,* sterilising females and eventually killing the weevil upon the emergence of a parasitoid larva. Between 1991 and 1998, *M. hyperodae* was widely released in the North Island with more limited releases in the South Island (McNeill *et al.* 2002). The release of *M. hyperodae* occurred at a time when detrimental livestock welfare issues associated with wild-type endophytes were becoming more unacceptable to the farming community.

The ideal outcome in any classical biological control programme is that pest numbers are reduced to a level whereby any damage is below an economic threshold, although in the case of *L. bonariensis* this has been difficult to establish. While the introduction of the related parasitoid *M. aethiopoides* Loan (Hymenopotera: Braconidae) was singularly successful in reducing damaging populations of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) (Goldson *et al.* 1992), in the case of *M. hyperodae*, the expectation was that biological control in conjunction with plant resistance would form part of an integrated pest management approach to managing *L. bonariensis*. Long term research at a release site near Lincoln in the South Island, demonstrated that the parasitoid had caused a significant reduction in first generation adult numbers (Goldson *et al.* 1998), but did not determine if biological control improved the persistence of the endophyte-free ryegrass in the pasture. North Island research showed similar numerical impacts, with life-tables analyses suggesting a c. 30% decline in adult numbers as a direct result of biological control (Barker & Addison 2006).

The advent of new endophytes, with little or no animal toxicity but repellency to *L. bonariensis*, provided farmers with a new opportunity to improve animal productivity utilising the qualities of ryegrass (Easton *et al.* 2001; Easton & Tapper 2005). However, while perennial ryegrass containing AR1 endophyte now form the majority of proprietary seed sown, short-lived Italian and hybrid ryegrasses (*L. multiflorum, L. boucheanum*), that are generally endophyte-free, are also utilised in pasture production. For these ryegrasses, reducing attack from *L. bonariensis* is dependent on biological control by *M. hyperodae*.

In 2003, a MAF Sustainable Farming Fund trial was established in the Wairarapa region of New Zealand to investigate the performance of ryegrass in an area where *M. hyperodae* had been present since releases in August of 1998. Wairarapa had traditionally been an area where ryegrass yield losses to *L. bonariensis* were significant and rapid (Kain *et al.* 1977). The main aims of the project were to obtain information from commercial farming operations on plant performance of, and animal growth rates on, new ryegrass-endophyte cultivars. The possibility that the persistence of low endophyte ryegrass had improved in the presence of *M. hyperodae* was also investigated.

Materials and Methods

Field sites

The research sites were established in autumn 2003 on four farms in Wairarapa (Table 1). On each farm, four treatments were established consisting of perennial ryegrass (cv. Aries) with either the wild-type or AR1 endophyte, or endophyte-free (nil), and a tetraploid ryegrass (cv. Quartet) with AR1. 'Wild-type' endophyte is the endemic endophyte strain of this cultivar, and through secondary metabolite production (alkaloids) will typically cause ryegrass staggers and reduce animal productivity. AR1 had been inoculated into this cultivar, and, with a different alkaloid profile to wild-type, does not have the same detrimental effects on livestock. Both endophytes produce peramine, the main alkaloid that protects endophyte-infected cultivars from damage caused by *L. bonariensis*.

Table 1 Site details for the four study sites in Wairarapa.

Site	Grid reference	Date sown	Soil type
1	41.11448S, 175.56308E	24 Mar 03	Kokotau silt loam
2	41.16807S, 175.25028E	12 Apr 03	Kohinui stony loam/ Opaki brown stony loam
3	41.14146S, 175.53227E	15 Mar 03	Wharekaka mottled fine sandy loam
4	41.08661S, 175.40536E	22 Mar 03	Tauherenikau stony silt loam

At each site, each treatment was allocated to a paddock with a minimum area of 2.3 ha. Sites 1, 3 and 4 were dryland sheep and beef farms and Site 2 a dairy farm. At Sites 1-3, paddocks were fully cultivated then sown, while at Site 4 the pasture was double sprayed with glyphosate then direct drilled. Fertilisers were applied to the paddocks at establishment. Sowing rates for cv. Aries was 16 kg/ha and for cv. Quartet ryegrass 21 kg/ha. All treatments included white clover (*Trifolium repens*) cv. Challenge (3 kg/ha) and cv. Tahora (1 kg/ha). Throughout the course of the study, pasture and stock management was left to the discretion of the farmers. On-farm trials to compare the performance of stock on the four treatments were carried out between the summer of Year 1 and autumn Year 2, but are not reported here. *Microctonus hyperodae* was released at 12 locations in Wairarapa in 1998 (McNeill *et al.* 2002) and a survey undertaken in 2002 showed that the parasitoid was established on all four farms. Endophyte contamination was measured in the AR1 treatments. The insect component assessed predominantly adult *L. bonariensis* and parasitism by *M. hyperodae*.

Ryegrass tiller populations

Ryegrass tiller populations were determined in spring (October) of Years 1, 2 and 3 and in autumn (April) of Years 2 and 3. Within each paddock, 15 x 75 mm diam. cores were taken along a diagonal transect across each paddock with subsequent sampling following a similar route to ensure that cores were collected from the same part of the paddock. Cores were dissected and the number of ryegrass tillers counted.

Endophyte presence and type

The first sampling for endophyte was first carried out in early summer of Year 1 (16 December 2003), with all 16 paddocks sampled. In summer Year 2, all four treatments from Site 1 were sampled on 16 December 2004 and all treatments except Quartet AR1 were sampled on the remaining sites on 6 January 2005. In mid-summer Year 3 (24 January 2006), all endophyte treatments at Sites 1, 3 and 4 were sampled, with Site 2 sampled in late winter Year 4 (12 August 2006). Since the change in endophyte infection rate from samples taken from Site 2 in August 2006 did not differ significantly from those sites sampled in January 2006, the results were still categorised as Year 3.

Within each paddock, samples were collected across a single transect with 6-10 ryegrass tillers randomly selected around c. 12 collection points. The presence of endophyte was checked using a tissue print immuno-blot assay (Hahn *et al.* 2003). Contamination of AR1 paddocks with wild-type endophyte was determined by testing endophyte-infected tillers for the presence of the endophyte alkaloid lolitrem B, produced by the wild-type but not AR1. A 3 cm section from the base of each tiller was freeze-dried and analysed for lolitrem B by HPLC (Gallagher *et al.* 1985) as outlined by Bluett *et al.* (2004). For tillers taken in Years 3 and 4, lolitrem B detection was determined using ELISA as described by Garthwaite *et al.* (1993) with modifications by Briggs *et al.* (2007).

Insects

Adult *L. bonariensis* were collected in spring (October) and autumn (April) of Years 1, 2 and 3, making a total of six collections. A further collection was carried out in late autumn Year 3 (May 2005) to determine the level of parasitism in the overwintering *L. bonariensis* population. The spring collection represented the reproductive survivors of the over-wintered adult population while the autumn population represented the majority of the *L. bonariensis* population recruited over the spring and summer prior to going into winter. The autumn population enters photoperiodically induced reproductive diapause and ceases egg laying until spring (Goldson & Emberson 1980).

Collections of *L. bonariensis* were made using a leaf blower vacuum (Echo 21 cc) which was used to suck the insects into a removable net recessed into the inlet pipe. Collections were made by dragging the blower vac along a 100 m transect from within each paddock. Weevils were removed from the litter, counted and parasitism levels determined either by rearing to emergence or dissection (e.g. McNeill *et al.* 2002). The use of the blower-vacuum did not allow for the determination of *L. bonariensis* density, but provided a relative score of adult numbers among farms and treatments. Other insect pests known to cause damage to ryegrass were also noted during the sampling.

Figure 1 Mean endophyte levels for the ryegrass-endophyte treatments measured across the four sites in Wairarapa between December 2003 and January 2006. Error bars represent the 95% confidence interval.

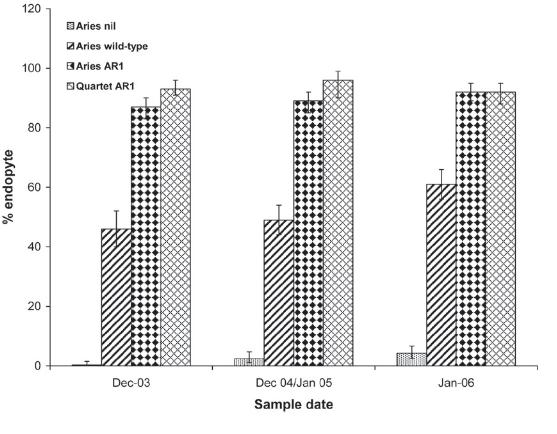

Table 2 Mean ryegrass tillers per m^2 for the ryegrass-endophyte treatments measured across four sites in Wairarapa in spring (October) and autumn (April).

Treatment	Spring Year 1	Autumn Year 2	Spring Year 2	Autumn Year 3	Spring Year 3	Overall mean	Linear trend	Spring - Autumn [1]
Log$_{10}$ means								
Aries nil	3.833	3.771	3.932	3.576	3.761	3.775	-0.068	0.168
Aries wild-type	3.744	3.897	3.942	3.662	3.842	3.823	-0.020	0.073
Aries AR1	3.789	3.893	3.974	3.629	3.767	3.810	-0.062	0.082
Quartet AR1	3.668	3.693	3.739	3.589	3.619	3.661	-0.041	0.035
LSD (5%)	0.195	0.122	0.164	0.261	0.223	0.088	0.162	0.169
Back-transformed means								**Spring / Autumn [2]**
Aries nil	6808	5902	8551	3767	5768	5957		1.47
Aries wild-type	5943	7889	8750	4592	6950	6653		1.18
Aries AR1	6152	7816	9419	4256	5848	6457		1.21
Quartet AR1	4656	4932	5483	3882	4159	4581		1.08

[1] The average of the three spring measurements minus the average of the two autumn measurements provides an indication of seasonal changes in ryegrass density.
[2] Calculated from the average of the spring measurements divided by the average of the autumn measurements.

Meteorological data

Total monthly rainfall and daily air mean temperature data were obtained from NIWA using meteorological stations based near Masterton, Te Ore Ore (station D05973) and East Taratahi AWS (station D15064).

Statistical analysis

Ryegrass tiller densities were log$_{10}$ transformed and analysed by ANOVA assuming a randomised complete block design by treating the four farms as four blocks and the four ryegrass–endophyte combinations as four treatments. The mean tiller density over time, the linear trend in tiller densities with time and the average of spring (3) versus autumn (2) densities were calculated (all on the log$_{10}$ scale) for each ryegrass-endophyte treatment, and these three variables were also subjected to analysis of variance.

Adult *L. bonariensis* numbers were analysed using a general linear model with a Poisson distribution and log link function. The relationship between adult numbers and percentage endophyte was determined by simple linear regression. Levels of parasitism among sites and ryegrass-endophyte treatments were compared by generalised linear model (GLM) procedure with a logit function and binomial error term (McCullagh & Nelder 1983). A regression was also carried out to determine the relationship between levels of parasitism in adult *L. bonariensis* collected in May 2005 and the percentage of endophyte-infect tillers. Differences in proportions of endophyte-infected tillers were assessed by calculating 95% confidence intervals on the binomial data.

Results

Ryegrass tiller populations

On average, cv. Aries had 39% greater tiller populations than Quartet AR1 (P<0.01) (Table 2), although this was not significant at all dates. Aires wild-type and AR1 populations were 12% and 8% higher respectively than Aries nil, but this was not significant at any date. There was a general decline in tiller densities over time for all treatments (P<0.10) but no significant difference among treatments (Table 2). Overall, tiller densities were significantly higher in spring than autumn (P<0.01) (Table 2), though the only treatment to show a significant seasonal effect was Aries nil.

Endophyte

The percentage of endophyte-infected tillers in AR1 paddocks was high on all farms at most assessments (Fig. 1), with a mean of 89% in Aries AR1 paddocks and 93% in Quartet AR1 paddocks. In contrast, infection rates for Aries wild-type paddocks averaged only 52% with significant differences among farms (range 46% to 60%). Over all sites, there was a significant increase with time in percent tillers infected with wild-type , (46%, 49% and 61% for Year 1, 2 and 3, respectively) (Fig. 1).

For Aries nil endophyte there was only a modest increase in endophyte levels over the study period (Fig 1.). There was a significant site effect, with Site 1 showing a mean endophyte level of 5%, while the ryegrass was free of endophyte at Site 2 and <1% at Site 3. At Site 4 the ryegrass was free of endophyte in Years 1 and 2 but in Year 3, 13% of tillers were infected.

Tests for lolitrem B in endophyte-infected tillers in Year 1 showed there was no or ≤ 2% contamination with wild-type endophyte in Aries AR1 and Quartet AR1 paddocks at Sites 2-4. In contrast, at Site 1 in Year 1, 35% of the endophyte-infected tillers from the Aries AR1 paddock and 14% of tillers from the Quartet AR1 paddock contained wild-type endophyte. Measurement of these paddocks in Years 2 and 3 showed no significant increase over time (3-year mean of 34% and 23% for Aries AR1 and Quartet AR1 paddocks, respectively).

Table 3 Mean number of *L. bonariensis* ± SE adults collected from the ryegrass-endophyte combinations in spring and autumn across the four sites in Wairarapa between October 2003 and April 2005 and associated parasitism by *M. hyperodae* (± SE).

	No. of *L. bonariensis*		% Parasitism	
	Spring	Autumn	Spring	Autumn
Treatment				
Aries nil	50.8±11.56	186.8±27.21	12.6±5.18	27.9±3.15
Aries wild-type	35.4±9.67	194.1±27.7	9.9±5.17	39.8±3.46
Aries AR1	22.8±7.76	24.3±9.80	5.1±4.62	36.1±8.81
Quartet AR1	25.1±8.14	28.0±10.54	3.7±3.92	50.8±8.56
Site				
1	129.4±14.32		30.1±3.35	
2	31.8±7.09		24.2±5.47	
3	63.9±10.06		36.5±5.20	
4	28.7±6.74		23.0±6.60	

Insects

L. bonariensis adult numbers were highest in the nil and wild-type endophyte pastures and lowest in the AR1 pastures (Table 3). Season had the most significant influence on adult numbers (F=52.15, P<0.001) followed by ryegrass-endophyte treatment (F=21.27, P<0.001) and site (F=20.16, P<0.001). There was also a significant but weak season by treatment interaction (F=4.18, P=0.01), due to a 4-5 times increase in adult numbers in the nil and wild-type pastures between spring and autumn, compared to little change in the AR1 pastures over the same period (Table 3). There was a significant negative relationship between the square root of *L. bonariensis* numbers in autumn and increasing endophyte levels (% variance accounted for = 21.2, P=0.007). There was no significant relationship between spring *L. bonariensis* adults and endophyte.

Other ryegrass insect pests observed in localised infestations during the course of the study were grass grub (*Costelytra zealandica*) (White), porina (*Wiseana* spp.) and pasture mealybug (*Balanococcus poae*) (Maskell). *C. zealandica* was only a problem at Site 2 where it caused sporadic damage to Aries-AR1 pasture.

Parasitism

Mean rates of parasitism in *L. bonariensis* in spring (8.7%) were significantly lower than found in autumn (35.2%) (χ^2 = 35.15, P<0.001). Rates of parasitism were highest in Aries wild-type and less in Aries AR1 pastures (Table 3) but the differences for the ryegrass-endophyte treatment were not significant. Mean levels of parasitism did not differ significantly among different ryegrass-endophyte treatments nor among sites (P>0.1). The levels of parasitism in autumn weevil populations were probably conservative, as parasitism rates tend to increase going into winter. This was reflected in a sample taken in May 2005, where there was a general increase in parasitism at all sites over the April 2005 sample (data not shown) and where parasitism in some of the nil and wild-type ryegrass pastures was >90%. Regression of percentage parasitism against percent endophyte for May 2005 showed only a weak relationship (% variance accounted for = 14.2%, P=0.09).

Meteorological data

Rainfall over the period March to May 2003, during pasture establishment, was c. 47% of the long-term monthly average rainfall. Thereafter, rainfall was near to, or greater than, the average monthly rainfall. In the summer period of December-February 2003-04, rainfall was on average 200% of the long-term mean while for the December-February 2004-05 period, rainfall was close to the long-term mean rainfall. Mean temperature over the same period were only noticeably higher in 2005.

Discussion

There was wide variation in adult *L. bonariensis* numbers across the four sites, with site 1 generally supporting the highest number of weevils irrespective of endophyte status. This probably reflected the northerly aspect of the trial site and associated warmer temperatures. Numbers were significantly lower in the ryegrass cultivars containing AR1, and there was clear evidence that population recruitment between spring and the subsequent autumn was a lot lower in these pastures. Adult numbers in April (autumn) appeared to be related to the proportion of endophyte-free ryegrass in the pasture, highest in the nil and wild type treatments and lowest in the AR1 ryegrass treatments. The relatively higher numbers of *L. bonariensis* on wild-type most probably reflected the moderate proportion of endophyte-infected tillers (52%) in these pastures, as AR1 and wild-type infected ryegrass tillers provide an equivalent high level of feeding deterrence to *L. bonariensis* adults through the production of peramine (Popay *et al.* 2003). Furthermore, ryegrass densities measured in this study were far higher than that measured by Barker *et al.* (1989) in Waikato, but similar to those measured under irrigated pasture in Canterbury (Goldson *et al.* 1998, McNeill *et al.* 2001). Therefore, it is highly probable that in the Wairarapa wild-type pastures, endophyte-free tillers were not limiting to *L. bonariensis* oviposition and subsequent adult recruitment.

While the increase in the proportion of wild-type infected tillers in our Wairarapa study is in keeping with the general phenomena of endophyte-infected tillers being favoured in New Zealand pastures, increases were moderate indicating only relatively mild selection pressures at these sites and years. The 11% difference (although not statistically significant) in tiller populations of between Aries nil and endophyte-infected (AR1 and wild-type), was also in favour of the endophyte-infected ryegrasses. In addition to this, there was no change in the proportion of wild-type endophyte contaminating AR1 pastures at Site 1. Hume & Barker (2005), summarising reported changes in endophyte levels over time in New Zealand pastures, highlighted the fact that changes over time are not consistent. For example, some sites with a low proportion of tillers infected with endophyte maintained a constant low status while in others there was a significant and rapid increase over time.

In New Zealand, changes in ryegrass persistence and proportions of endophyte-infected tillers are usually closely linked with the severity of insect damage (e.g. *L. bonariensis*) and degree of drought stress in summer/autumn. In the past, *L. bonariensis* has been a key insect pest driving these changes (e.g. Kain *et al.* 1977; Francis & Baird 1989). In field studies on the impact of biological control, Goldson *et al.* (1998) and Barker & Addison (2006) reported declines in *L. bonariensis* populations over time. In particular, Goldson *et al.* (1998) noted that at a site in Canterbury, the more damaging first summer generation *L. bonariensis* larval peaks declined commensurate with a build-up in parasitism, leading to the suggestion that *M. hyperodae* was significantly reducing adult densities. Furthermore, low adult numbers were observed in a study examining the relationship between endophyte, *L. bonariensis* and its parasitoid *M. hyperodae* (Goldson *et al.* 2000), leading to the suggestion that parasitism may have suppressed weevil numbers. McNeill *et al.* (2001) reported a similar result on a dairy farm near Lincoln, where adult numbers were high in the first summer following pasture establishment (c. 128 /m^2) but thereafter, were less than 40 /m^2.

When conceived, the main aims of this project were to obtain information on the plant performance and animal growth rates that could be expected with the new ryegrass-endophyte cultivars. It also provided an opportunity to determine if the persistence of low endophyte ryegrass had improved in the presence of *M. hyperodae*, the implication being that the parasitoid had perhaps reduced the damage caused by *L. bonariensis*. The four sites used in this study covered a relatively wide range of environments but a common theme was that the nil-endophyte pastures were maintaining their generally low endophyte levels despite supporting higher *L. bonariensis* populations when compared with numbers found in the AR1 pastures. Similarly, in the wild-type ryegrass pastures there was not the rapid increase in endophyte status nor tiller loss that perhaps could have been expected to occur as a result of *L. bonariensis* larval damage in the drier east coast regions of New Zealand in the past (e.g. Kain *et al.* 1977; Francis & Baird 1989). However, these results showed that in some environments (Site 1), an increase in endophyte levels was associated with high numbers of *L. bonariensis*. Interestingly, this was in Aries wild-type endophyte where between Dec 2003 and Jan 2006 endophyte levels increased significantly from 51 to 70%. In the nil-endophyte pasture at Site 1, there was no significant change with values of 1 and 4% recorded from tillers taken over the same time period. Obviously, *L. bonariensis* is not the only insect that favours endophyte-free ryegrass, with pasture mealybug present at some of these sites and likely to have been providing further selection pressure for endophyte-infected tillers (Pennell *et al.* 2005).

Biological control of *L. bonariensis* may be successful in improving persistence of endophyte-free pastures in regions of New Zealand where the environment is favourable to the parasitoid and particularly where attack from other pests such as black beetle, pasture mealybug or porina are sporadic or absent. Biological control can also has a positive interaction in high-endophyte pastures, with parasitism of adult *L. bonariensis* in spring reducing oviposition on endophyte infected ryegrass at a time when the peramine concentrations in the leaf lamina are low and the plant is more susceptible to attack (Popay & Mainland 1991; Ball *et al.* 1991). In conclusion, it is possible that in regions such as Wairarapa and Canterbury biological control by *M. hyperodae* may attenuate the damage caused by *L. bonariensis* and therefore extend productivity and survival of ryegrass pastures where *L. bonariensis* is the key pest.

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