Damage potential of Argentine stem weevil in Lincoln dairy pasture: has biological control by *Microctonus hyperodae* altered the balance?

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Abstract

To measure the impact of biological control of Argentine stem weevil, *Listronotus bonariensis* (ASW) by *Microctonus hyperodae* in dairy pasture, a research site was established in the autumn of 1997 on a dairy farm near Lincoln, where the parasitoid had been present since 1994. Existing pasture was killed with glyphosate and four ryegrass cultivar treatments consisting of three perennial (Nui, Embassy, Vedette) and one hybrid mixture (Marsden/Greenstone), each containing either low or high levels of *Neotyphodium lolii* endophyte, were sown by direct drilling. A third main plot treatment was low endophyte plus insecticide. Between 1997 and 1999, seasonal pasture production was measured in all plots. Herbage composition was also measured in all plots for the first 2 years but only in the Nui treatments in 1999. ASW adult and larval densities, larval damage to ryegrass, parasitism by *M. hyperodae* and ryegrass tiller density were measured on Nui plots in spring and again in summer, coinciding with periods of peak ASW egg laying and larval development.

Between 1997 and 2000, ASW adult densities in Nui were highest in the first summer (26 January 1998) following establishment. Thereafter, densities did not rise above c. 60 ASW/m². Densities of adults, larvae, levels of larval damage to ryegrass, and parasitism by *M. hyperodae* were significantly higher in summer than spring (P<0.001) but with the exception of larval damage, there were no significant differences between the low and high endophyte Nui. There were no significant differences in herbage production among the low endophyte, high endophyte, and low endophyte plus insecticide treatments for any of the ryegrass cultivar treatments. The ryegrass component of the pasture showed a similar rate of decline over time for all Nui treatments. Over a 20-month period, average levels of endophyte in the high endophyte Nui plots increased from 35 to 46%. Conversely, average levels in the low-endophyte Nui plots, remained at c. 5%. The lack of any significant differences in herbage production and the relatively stable endophyte levels are discussed in relation to the ASW population data and *M. hyperodae* parasitism rates obtained from the Nui plots.

Keywords: Argentine stem weevil, biological control, dairy pasture, endophyte, *Lolium perenne*, *Microctonus hyperodae*, ryegrass cultivars

Introduction

Argentine stem weevil, *Listronotus bonariensis* (ASW), has historically been a major pest of ryegrass in Canterbury (Pottinger 1961; Trought 1976; Barker *et al.* 1981), and one that has been difficult to control (Goldson *et al.* 1990). In an effort to broaden the options for control of ASW, the Braconid parasitoid *Microctonus hyperodae* Loan was imported into New Zealand quarantine as part of a classical biological programme against the pest (Goldson *et al.* 1990). Following approval from MAF, the parasitoid was first released in 1991 at nine locations throughout New Zealand (Goldson *et al.* 1993). The parasitoid established and built up at the majority of the release sites (Goldson *et al.* 1993; 1994), with commercial releases made at a further 111 locations in the North Island and Canterbury between 1994 and 1998 (unpublished data). An intensive study conducted at the original Lincoln release site provided valuable information on the impact of the parasitoid on the ASW population (Goldson *et al.* 1998a, b), as well as details on the rate of dispersal of the parasitoid from the release paddock (Goldson *et al.* 1999). However, despite evidence of suppression of ASW egg and larval populations (Goldson *et al.* 1998a), no conclusions could be drawn as to whether suppression of weevil populations was reflected in improved ryegrass production.

This study attempted to answer this question by investigating pasture production in relation to a selection of ryegrass cultivars, *Neotyphodium lolii*-endophyte levels, and ASW adult and larval populations in the presence of the parasitoid *M. hyperodae*.

Materials and methods

Preparation and maintenance of the research site
The site was on a dairy farm located 2.5 km from the AgResearch Lincoln release site. *Microctonus hyperodae* was known to have reached the farm between the winter of 1993 and July 1994 (Goldson *et al.* 1999), and by 1997 was widely distributed around the Lincoln area. The existing ryegrass–white clover pasture was grazed then sprayed with 4.5 l/ha glyphosate herbicide on 15 February 1997 and the plots direct drilled on 12 March 1997. One hundred kg/ha of superphosphate and 10 kg/ha of slug bait were drilled with the pasture mixes. Forty-five kg/ha of urea fertiliser was applied on 10 April 1997. There were 12 treatments consisting of three perennial ryegrass cultivars (Grasslands Nui, Embassy, Vedette) and a hybrid ryegrass mix (Grasslands Marsden/ Grasslands Greenstone) containing either high *N. lolii* endophyte levels, low *N. lolii* endophyte levels or low *N. lolii* endophyte levels with insecticide. The insecticide treatment was included to determine whether chemical control of ASW improved herbage production in the low endophyte plots. Each treatment was replicated three times giving a total of 36 plots. Plots were approximately 25 m x 30 m arranged in a randomised block design. The Nui, Embassy, and Vedette lines were sown at 20 kg/ha and the Marsden/ Greenstone mixture at 18 kg/ha (8kg/ha Marsden, 8 kg/ha Greenstone, and 2 kg/ha of Grasslands Kahu timothy). All lines were sown with 3 kg/ha of Grasslands Kopu white clover and 2 kg/ha Grasslands Pawera red clover.

The research site was used as a night paddock, providing 3 days grazing with approximately a 16-day rotation starting in August of each year and carrying through to the following May. As required, irrigation commenced late-October of each year and continued through to mid-April of the following year. The pasture was irrigated as soon as grazing was completed with a travelling-gun irrigator applying approximately 50 mm of water/application. Pasture was topped as part of the general management of the farm. Fertiliser was applied in November 1998 with 200 kg/ha of ammonium sulphate, DAP (18% N, 20% P, 2% S), and KCl. In February 1999, a 125 kg/ha mix of Cropmaster 15 (15% N, 10% P, 10% K, 8% S), DAP and KCL was applied.

Insecticide application
Chlorpyrifos was applied at 500 g ai/ha two to three times between September–November to kill the overwintering ASW adults and those adults that may have immigrated from adjacent plots. Two applications

were also made in summer (late December–January) to kill any adults that may have arisen from egg laying by weevils that had survived the insecticide applied in spring. Sampling of the low endophyte Nui plus insecticide plots in the first spring and summer of the study (1997–98) showed that this regimen maintained ASW egg and larval populations at generally lower levels than the untreated plots (unpublished data). It was assumed that the ASW populations in the insecticide plots would be sufficiently controlled so as not to cause any significant yield losses and these plots were not sampled for the rest of the study.

Pasture mass
Pasture mass levels were measured pre- and post-grazing using a pasture probe. Calibration cuts were made four times/year (spring, summer, autumn, winter). Twelve dry matter assessments were made between 30 August 1997 and 18 May 1998, with a further 10 and 9 assessments made between 14 September 1998– 12 May 1999 and 14 September 1999–26 April 2000 respectively.

Pasture composition assessment
Pasture composition was assessed at irregular intervals by taking 25 small samples per plot and bulking each of the three replicates per treatment. Dissections recorded the percentage of (1) ryegrass, (2) timothy, (3) clover species (white and red), (4) other grasses, (5) weeds, and (6) 'dead'. Pasture composition cuts were made on 17 October 1997, 23 February 1998, 8 May 1998, 11 November 1998, and 13 January 1999. On 28 April 2000, only Nui treatments were sampled.

Grasslands Nui tiller density
Grass tiller densities were measured by taking 62-mm-diameter cores to a depth of 30 mm. In the first sample (20 October 1997), only 10 cores were taken; thereafter 15 cores were taken from each plot. Cores were dissected, grass species were identified and numbers of ryegrass tillers counted.

Ryegrass endophyte level
Endophyte levels in the Nui plots were measured by randomly selecting c. 100 ryegrass tillers from within each plot and determining their endophyte status using either the immunoblot technique (Gwinn *et al.* 1991) or ELISA (Miles *et al.* 1998). Samples were taken on 5 March 1998, 7 December 1998, and 6 December 1999. Endophyte levels in the other cultivars were not routinely measured in this study, but a single assessment was taken on 5 March 1998 from the Vedette and Marsden/Greenstone plots. Mean endophyte levels for

the low and high endophyte Vedette were 97 and 53% respectively, and for low and high endophyte Marsden/ Greenstone plots, 6 and 88% respectively.

Argentine stem weevil population dynamics
ASW population measurements were restricted to the low and high endophyte Nui treatments. Plots were sampled at key times of the ASW life cycle in spring (September–November) and again in summer (January– March) to measure oviposition activity and ensuing larval densities.

ASW adults
Adults were sampled once in spring and again in summer. The spring sample measured the overwintered adult population from which the spring egg and larval populations arose and was taken on 20 October 1997, 14 October 1998, and 12 October 1999. The summer adult sample measured the first summer generation prior to any significant oviposition. The summer adult samples were taken on 26 January 1998, 25 January 1999, and 31 January 2000. Adults were sampled by taking twenty 450 mm x 40 mm x 80 mm deep (0.018 m^2) rectangular turves and extracted using a flotation method described by Goldson *et al.* (1998a).

ASW larvae and tiller damage
Larvae were sampled by randomly removing tillers from within the plots at fortnightly intervals using a knife to cut tillers at ground level. In the laboratory, c. 110 ryegrass tillers were selected and examined for larval feeding damage. Tillers were sampled in the spring of 1997 on 20 October, 3 November, and 17 November; spring of 1998 on 12 October, 27 October, 9 November, and 23 November; and spring of 1999 on 12 October, 26 October, 8 November, and 22 November. Tiller sampling in the summer of 1998 was on 27 January and 10 February, and summer of 1999 on 25 January, 8 February, 22 February, and 8 March. ASW larval density was calculated as the number of larvae recovered/ number of tillers examined multiplied by tiller density.

Parasitism by M. hyperodae
To determine parasitism levels, ASW adults were collected using a commercial leaf vacuum machine (McCulloch SuperAirsteam III®, 21.2 cc) with a net fitted to the inlet nozzle. Collections generally coincided with ASW larval and adult sampling. Weevils were extracted from the litter and their level of parasitism was assessed by either rearing parasitoids from the weevils (Goldson & Proffitt 1991) or by dissecting the weevils under a dissecting microscope. Parasitism levels were determined in ASW adults collected in the spring

of 1997 (20 October, 3 November, and 17 November), 1998 (14 October, 27 October, 9 November, and 23 November), and 1999 (12 October, 26 October, 8 November, and 22 November). In the summer, parasitism levels were ascertained from adults collected in 1998 (26 January, 9 February, and 3 March) and 1999 (25 January, 8 February, 22 February, 8 March, and 12 April).

Statistical analysis
Dry matter yields were analysed using ANOVA for a randomised block design and comparing (a) ryegrass cultivars, (b) endophyte and insecticide treatments and (c) the interaction between the two. Ryegrass tiller densities, ryegrass % in the pasture, ASW adult densities and larval densities were analysed using ANOVA. Endophyte levels, percentage of tillers damaged by ASW larvae and parasitism rates were analysed using the Generalised Linear Model (GLIM) procedure (McCullagh & Nelder 1983). Analyses examined the main effects and interactions of endophyte (low and high levels), season (spring and summer) and year (endophyte only). All means are presented as means ± SEM. Analyses were carried out using the Genstat statistical program.

Results

Pasture mass
There were no significant differences in pasture mass between cultivars, endophyte, and insecticide for each of the 1997–98, 1998–99, and 1999–00 grazing periods (Table 1). For the 1997–98 grazing period, there were no significant main effects or interactions in any of the 12 DM measurements taken between 30 August 1997 and 18 May 1998. In 1998–99, only the first measurement (19 August 1998) showed that Nui had a significantly lower DM yield than Vedette (P<0.05). Similarly, in 1999–2000 there were no significant cultivar, endophyte or low endophyte plus insecticide treatment effects, except for the last sample (26 April 2000), where there was a significant cultivar effect between the Marsden/Greenstone mix and Embassy (P<0.05).

Pasture composition
In the 1997–98 season, mean ryegrass pasture composition levels across the four cultivar selections were c. 74% (after excluding the 'dead' component), but by the second year ryegrass content in all treatments, with the exception of the high endophyte Nui, had declined to a mean of c. 64% (Table 2). In the final cut on 28 April 2000, the ryegrass component for the three Nui treatments had declined further,

but to a similar extent in each treatment (Table 2). The decline in the ryegrass component was associated with an increase in the 'clover' and 'other grass' components of the sward (data not presented). Overall, there was a significant decline in the ryegrass component of the pasture over time (P<0.001) and a significant cultivar effect (P<0.001). However, there was no significant time x cultivar effect (P>0.5), nor were there any significant differences among the high, low, and low endophyte plus insecticide treatments (P>0.2). The cultivar x endophyte/low endophyte plus insecticide interaction was significant (P<0.05).

Grasslands Nui tiller density

Overall, ryegrass tiller densities were significantly higher (P<0.01) in summer than in spring (Table 3). The high endophyte plots had higher overall tiller densities than the low endophyte plots (Table 3) but this was not significant (P>0.2). The season x endophyte interaction was significant (P<0.05).

Endophyte

Overall, the mean percentage endophyte for the high endophyte plots increased by c. 10% between 1997–1999 (Table 4). By comparison, endophyte levels in the low endophyte treatments were relatively stable at c. 5% for the duration of the study (Table 4). While there was a significant difference in endophyte levels between the low and high endophyte treatments (P<0.001), there was neither a significant year effect (P>0.2), nor a significant endophyte x year interaction (P>0.5).

Argentine stem weevil

ASW adults

Adult densities measured in the low endophyte treatments were generally similar to those in the high endophyte treatment (Figure 1). Spring adult numbers were generally highest in the first year of establishment averaging 18.5 ± 4.2 and 21.3 ± 4.5 adults/m^2 in the low and high endophyte plots respectively. In the springs of 1998 and 1999 densities were generally lower, with only one endophyte replicate exceeding 20 adults/m^2 in 1998. Summer adult numbers were highest in the first summer (January 1998) with densities generally exceeding c.128 adults/m^2. Thereafter, summer peaks generally remained below 40 adults/m^2. Overall, mean densities in the spring and summer populations were 12.5 ± 1.6 and 63.3 ± 3.6 adults/m^2 respectively. Mean overall densities in the low

Table 1 Seasonal dry matter production (kg DM/ha) at the Lincoln dairy farm for 1997–98, 1998–99 and 1999–2000 for the five ryegrass cultivar treatments, and endophyte-insecticide treatments. Differences in dry matter production among cultivars and endophyte/insecticide treatments were non-significant for all three seasons.

Season	Cultivar	Endophyte Low	High	Low endophyte + Insecticide
1997–98	Nui	8780	9620	8920
	Embassy	10260	10360	9650
	Vedette	9240	9600	10520
	Marsden/ Greenstone	10430	10000	10070
	SEM	632		
1998–99	Nui	10240	10520	9360
	Embassy	11380	10530	9810
	Vedette	10740	10790	11620
	Marsden/ Greenstone	11080	10160	10900
	SEM	719		
1999–2000	Nui	11900	11790	10870
	Embassy	12470	12270	10780
	Vedette	11080	11550	12860
	Marsden/ Greenstone	12650	10350	12760
	SEM	789		

Table 2 Percentage ryegrass component of the five ryegrass cultivar treatments between 1997 and 2000. Only the Grasslands Nui plots were sampled in 2000.

Treatments	Sample dates 1997/98 *	1998/99 **	28 April 2000
Low endophyte			
Nui	81.2	78.8	63.6
Embassy	74.9	70.9	
Vedette	75.6	75.3	
Marsden/Greenstone	69.6	60.6	
High endophyte			
Nui	78.9	86.1	62.3
Embassy	78.2	69.6	
Vedette	75.1	62.9	
Marsden/Greenstone	60.4	49.6	
Low endophyte plus insecticide			
Nui	75.2	56.4	65.1
Embassy	81.5	68.7	
Vedette	73.8	66.9	
Marsden/Greenstone	69.9	56.4	

Treatment	Cultivar	Endo/Insect.[1]	Cultivar x Endo/Insect.	Date
SEM	1.82	1.58	3.15	2.23
Significance	P<0.001	ns	P<0.05	P<0.001

* = mean of three sample dates, ** = mean of two sample dates
[1] Endo/Insect. = High, low and low endophyte plus insecticide treatments

and high endophyte plots were 40.0 ± 2.9 and 35.8 ± 2.7 adults/m^2 respectively and were not significantly different (P>0.2). Overall, there was a significant season effect (P<0.001), but no significant season x endophyte interaction (P>0.5).

Table 3 Mean Grasslands Nui ryegrass tiller density, ASW larval density and the percentage of tillers sustaining larval damage in the spring (1997, 1998, and 1999) and summer (1998 and 1999), and low and high endophyte plots.

	Season		Endophyte treatment	
	Spring	Summer	Low	High
Ryegrass density/m^2	3719	4515	3838	4210
SEM	188.2		145.1	
Significance	P<0.01		ns	
ASW larval density/m^2	61	134	101	75
SEM	14.1		14.4	
Significance	P<0.001		ns	
% ryegrass tillers damaged	4.6	9.2	7.5	5.0
SEM	0.25	0.46	0.36	0.29
Significance	P<0.001		P<0.001	

Table 4 Percentage endophyte levels in Grasslands Nui ryegrass in low and high endophyte plots measured on three dates.

	--------------- Sample date ---------------		
Endophyte treatment	5 Mar 1998	7 Dec 1998	6 Dec 1999
Low endophyte	5.0	4.7	5.3
SEM	2.1	1.2	1.4
High endophyte	34.8	40.0	46.2
SEM	2.7	2.8	3.2
Treatment	Date	Endophyte	
Significance	ns	P<0.001	

ASW larvae and tiller damage
Overall, for the entire period of the study, summer larval densities were significantly higher than spring densities (P<0.001) (Table 3). Densities in low and high endophyte plots were not significantly different (Table 1) (P>0.2), nor was there a significant season x endophyte interaction (P>0.5). Larval damage to ryegrass tillers also showed a similar seasonal effect with damage significantly higher in summer compared to spring (P<0.001) (Table 1). Low endophyte Nui sustained significantly higher larval damage than the high endophyte plots (P<0.001) (Table 3), but the season x endophyte interaction was not significant (P>0.05).

Parasitism by M. hyperodae
Levels of parasitism of ASW adults showed a general increase during each spring and summer sampling interval (Figure 2), consistent with the phenology of the parasitoid as measured at the Lincoln release site (Goldson *et al.* 1998b). Parasitism rates at the commencement of the experiment (20 October 1997) were only c. 1%, but this had increased to c. 22% by

Figure 1 Adult Argentine stem weevil (ASW) density measured in the low and high endophyte Grasslands Nui plots. The data sets are slightly offset for clarity. Bars indicate 95% confidence limits.
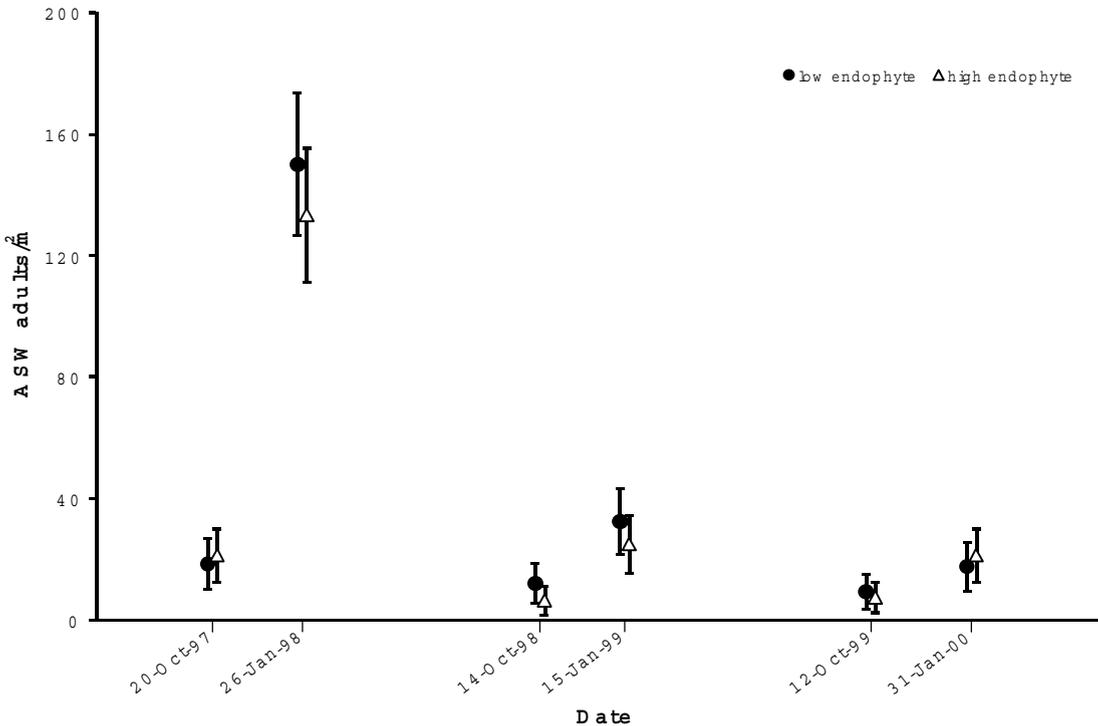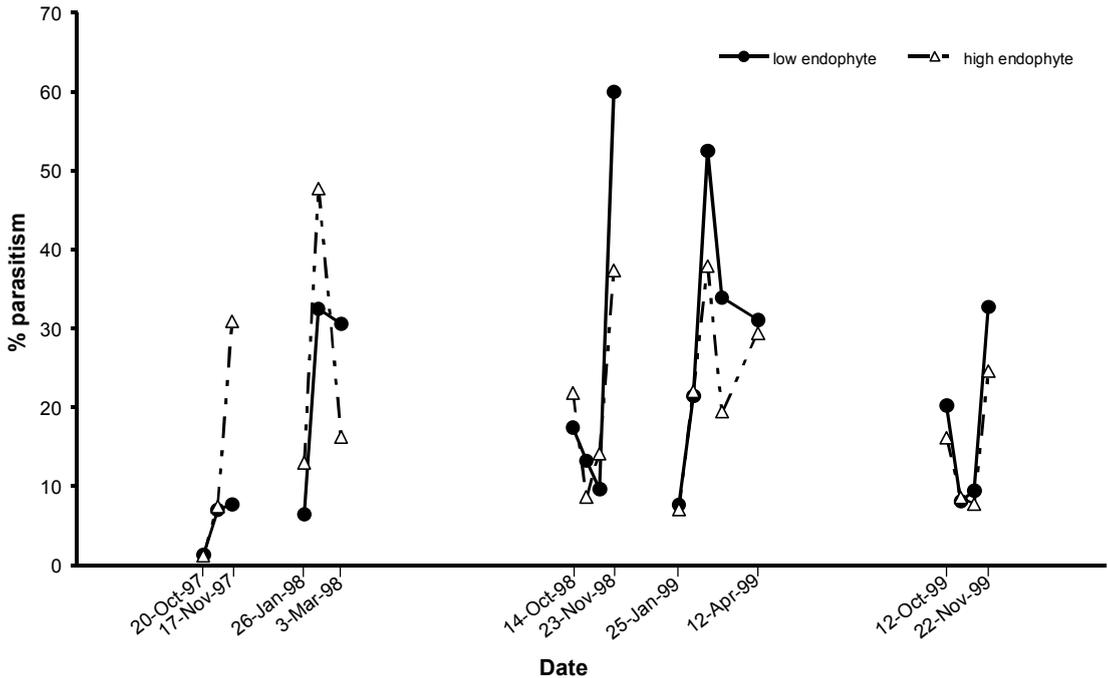
Figure 2   Percentage parasitism of Argentine stem weevil by *Microctonus hyperodae* in the low and high endophyte Grasslands Nui plots.

17 November 1997. Over the entire experiment period (1997–1999), the parasitism rate in spring and summer averaged 16.0 ± 2.0 and 27.3 ± 2.0% respectively and was significantly different (P<0.001) between the seasons. Mean levels of parasitism in the low and high endophyte plots were 24.2 ± 2.0 and 21.0 ± 2.0% respectively and not significantly different (P>0.2). There was no significant season x endophyte interaction (P>0.5).

Discussion

All ryegrass cultivars used in this study showed similar levels of production under dairying irrespective of endophyte status or insecticide application. While the pasture ryegrass composition showed a significant cultivar and time effect, the rate of decline in the ryegrass component was similar for all cultivars. Similar trends were evident in the more intensive ryegrass tiller study on Nui, which found no significant differences in tiller density between the low and high endophyte treatments.

With the exception of the first summer (1998), ASW adult and larval densities were generally low throughout the course of the study. While both adults and larvae showed significant seasonal differences there were no significant differences between the high and low endophyte Nui treatments. However, this is in

marked contrast to other studies. For instance, comparison between low and high endophyte pastures have generally shown that ASW cause significant yield losses in the former, even under condition of high nutrition and adequate moisture (Kain *et al.* 1982; Hunt *et al.* 1988; Francis & Baird 1989). Furthermore, this study did not find that the use of insecticide to control ASW lead to a significant improvement in yields in the low endophyte treatment. Previous studies have found a significant improvement in total herbage yields when insecticides have been used to reduce ASW larval populations (Kain *et al.* 1977; Prestidge *et al.* 1984).

Larval densities and the percentage of tillers damaged were generally higher in summer than spring. These results contrast with earlier studies in Canterbury, which found that the spring population was the most damaging (Pottinger 1961; Barker *et al.* 1981). This may be an indication of the impact of *M. hyperodae*. Lincoln studies of the bionomics of ASW pre- and post- *M. hyperodae* establishment showed that there was a reduction in the size of the first summer (spring) generation egg and larval peaks, such that the second generation became more important (Goldson *et al.* 1998a). This led the authors to suggest that this change in the relative importance of the two generations was a direct result of the parasitoid.

The relatively stable level of endophyte over the 20-month interval between the first and last endophyte measurements, especially in the low endophyte plots, is also in marked contrast to other studies. Research at AgResearch Winchmore between 1986–1988 found that endophyte levels in low endophyte Nui, growing under irrigation, increased rapidly over 3 years (Francis & Baird 1989). Studies in the central Volcanic Plateau recorded increases in endophyte levels in Nui of 33–400% over a 12-month period (Prestidge *et al.* 1984).

The low level of parasitism at the start of the study was probably a reflection of the disruption caused by the defoliation of the pasture prior to drilling the plots in March of that year. Suppression in parasitism owing to the use of glyphosate to kill off existing pasture has been observed at Ruakura (P. Addison, pers. comm.). Subsequently, there was a yearly increase in parasitism, similar to that observed at the AgResearch Lincoln research site (Goldson *et al.* 1998b).

The original aim of the research was to determine whether biological control of ASW by the parasitoid *M. hyperodae* was translated into improved ryegrass dry matter production and persistence under intensive dairying. This research found: (a) no significant differences in dry matter production and persistence between low and high endophyte treatments, (b) no differences in dry matter production and persistence between low endophyte ryegrass and low endophyte ryegrass treated with insecticide and (c) no significant increase in endophyte levels in Nui. This suggests that ASW larval and adult populations were operating below levels that lead to yield losses or rapid reversion to high endophyte. However, it is difficult to attribute low ASW population densities to regulation by *M. hyperodae*, as there is no parasitoid-free control against which these results can be compared. Nonetheless, recently published results from research sites near the original 1991 parasitoid release sites in Ruakura, Waikato and Lincoln, Canterbury provide anecdotal evidence of parasitoid-mediated suppression of ASW populations. Van Vught & Thom (1998) and Burggraff & Thom (2000) reported improved tiller survival, persistence and yield of low endophyte perennial ryegrass on a site at Ruakura. Research on a site at the AgResearch farm, Lincoln, found consistently low levels of larval damage in Nui (Popay *et al.* 1999).

Further work is underway to quantify parasitoid impacts through key factor analysis of data collected from the AgResearch Lincoln research site (S.L. Goldson pers. comm.). Until then, a definitive statement as to the success or otherwise of *M. hyperodae* is reserved. Notwithstanding any significant suppression of ASW populations by *M. hyperodae*, other ryegrass pests such as black beetle (*Heteronychus arator*) (Popay & Baltus 2001) and pasture mealy bug (*Balanococcus poae*) (Popay *et al.* 1999) will need to be considered in the decision to sow low-endophyte ryegrass cultivars.

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