New Zealand Journal of Agricultural Research, 2003, Vol. 46: 311–319 311
0028–8233/03/4604–0311 $7.00/0

Interactions between endophyte (*Neotyphodium* spp.) and ploidy in hybrid and perennial ryegrass cultivars and their effects on Argentine stem weevil (*Listronotus bonariensis*)

A. J. POPAY[1]
D. E. HUME[2]
K. L. DAVIS[3]
B. A. TAPPER[2]
[1]AgResearch
Ruakura Research Centre
Private Bag 3123
Hamilton, New Zealand
[2]AgResearch
Grasslands Research Centre
Private Bag 11 008
Palmerston North, New Zealand
[3]Dexcel
Private Bag 3221
Hamilton, New Zealand

Abstract Argentine stem weevil damage was assessed in 1998 and 1999 on two diploid perennial ryegrasses (cvs. 'Yatsyn' and 'Samson'), a diploid long-rotation hybrid ryegrass (cv. 'Marsden'), a tetraploid perennial ryegrass selection ('4N') and in a tetraploid hybrid (cv. 'Greenstone') in a field trial in Taranaki. 'Greenstone' was infected with a selected *Neotyphodium* endophyte that produces peramine and ergovaline but not lolitrem B. All other cultivars were naturally infected with endophytes that produce all three alkaloids. In the first year, percent endophyte infection levels were low in 'Greenstone' (61%), 'Marsden' (70%) and '4N' (71%) relative to 'Yatsyn' (85%) and 'Samson' (86%), but increased in the second year to greater than 88% in all cultivars. Argentine stem weevil adult feeding and larval damage were greatest on 'Greenstone' and least on 'Yatsyn' and 'Samson' in both years. 'Greenstone' tillers damaged by larvae were more severely affected than tillers of other cultivars. Individually and in combination, percent endophyte and peramine and lolitrem B levels in the leaf lamina were correlated with adult activity which in turn was the main factor affecting larval damage. Overall, the tetraploid and hybrid species sustained more damage than the diploids and perennials. This was attributed mainly to lower leaf lamina peramine concentrations in the hybrid and tetraploid combinations which affected adult feeding, but additional attractiveness factors in these cultivars may also play a role in their susceptibility to Argentine stem weevil. The absence of lolitrem B was not a major factor in the number of tillers damaged by larvae in 'Greenstone', but may have resulted in more severe damage to those tillers.

Keywords ryegrass; cultivar; ploidy; hybrid; perennial; endophyte; alkaloids; Argentine stem weevil; *Neotyphodium*; *Lolium*

A02063; Online publication date 5 November 2003
Received 14 October 2002; accepted 13 June 2003

INTRODUCTION

Argentine stem weevil (ASW) (*Listronotus bonariensis*) adults and larvae are highly responsive to different hosts and this is usually reflected in the amount of damage caused by the stem-boring larvae. Early work on ASW identified the particular susceptibility of the Italian (*Lolium multiflorum*) and short-rotation ryegrasses (*Lolium multiflorum* × *perenne*) to damage (Kelsey 1958; Whatman 1959; Hoy 1960). In a survey in Canterbury, 77% and 84% of tillers from Italian and short-rotation ryegrasses, respectively, were damaged by larvae compared with 35% of perennial ryegrass tillers (Timlin 1964). Another study found that the hybrid ryegrass 'Grasslands Ariki' ((*L. perenne* × *multiflorum*) × *perenne*) suffered similar damage to the perennial 'Grasslands Ruanui' and less damage than another hybrid 'Grasslands Manawa' (*L. perenne* × *multiflorum*) (Barclay 1963). In an extensive study, Goldson (1982) concluded that ovipositional preference and larval damage by ASW was related to the degree of annual parentage in the cultivar. Thus, the tetraploid annual 'Westerworlds' ryegrass (*L. multiflorum*), 'Grasslands Tama', and the diploid annual, 'Grasslands Paroa', were more susceptible than the hybrid 'Manawa', which in turn was more susceptible than 'Ariki'. All annual and hybrid cultivars were considerably more damaged than the two perennial cultivars 'Ruanui' and 'Nui'. Subsequent to Goldson's study, the important discovery that infection of ryegrass with endophyte (*Neotyphodium lolii*) protected their hosts from ASW damage was reported by Prestidge et al. (1982). With this discovery much of the early information on ASW host preference appeared to become obsolete since the endophyte status of the plants tested was unknown. However, a study by Barker (1989), showing that in the absence of *N. lolii* infection ryegrass cultivars of *L. multiflorum* parentage were preferred over perennial cultivars as hosts of ASW, confirmed some of the previous work. Popay et al. (1995) also found that, without endophyte, 'Grasslands Moata' (*L. multiflorum*) and 'Grasslands Greenstone' ((*L. perenne* × *multiflorum*) × *perenne*) were more susceptible to ASW than the perennial cultivars 'Nui' and 'Pacific'. Endophyte infection reduced damage to all cultivars, but 'Moata' and 'Greenstone' still sustained more damage than the perennials.

In 1997, a trial was established near Hawera in Taranaki to compare milk solids production on three diploid and two tetraploid ryegrass cultivars, all of which were infected with endophyte. An infestation of ASW in this trial in the summers of 1998 and 1999 provided an opportunity to further investigate the interactions between endophytes and their different ryegrass hosts with respect to ASW damage, including the effects of ploidy and ryegrass species.

METHODS AND MATERIALS

Site and treatments

The experiment was at the Westpac-Trust Agricultural Research Station in South Taranaki on high fertility (e.g., average Olsen P = 57 ppm) volcanic soils (Stratford sandy loam, Egmont brown loam). Nine replicates of each of the four ryegrass cultivars and one ryegrass selection were sown in 0.2-ha plots on 12 and 14 March 1997, following a summer forage crop. Plots were arranged in a randomised block design with each block assigned to nine different paddocks. Plots were sown at 15 kg (diploid cultivars), 25 kg (tetraploid cultivars) and 19.5 kg (tetraploid selection) of viable ryegrass seed/ha, and then maintained as pure ryegrass plots through the use of herbicides to control weeds and white clover. Plots were grazed by dairy cows as part of the normal rotation on the farm at a grazing intensity of approximately 150 cows/ha for a 12-h grazing period every 3–6 weeks during good growth periods, and once over the winter period with dry cows. Five applications of 50 kg N/ha in the form of urea (46% N) were made during the year to give a total annual application of 250 kg N/ha.

Of the five ryegrasses, two were diploids, cvs. 'Grasslands Marsden' and 'Grasslands Samson', with their respective tetraploids, cv. 'Grasslands Greenstone' and the selection '4N', together with a third diploid, cv. 'Yatsyn'. 'Marsden' and 'Greenstone' are long-rotation hybrid ryegrasses (*L. boucheanum* syn. *L. hybridum*) while 'Samson', '4N' and 'Yatsyn' are perennial ryegrasses (*L. perenne*). All but 'Greenstone' were infected naturally with endophytes which produce the alkaloids peramine, lolitrem B, and ergovaline. 'Greenstone' was infected with a selected endophyte which produces ergovaline and peramine but not lolitrem B.

Measurements

Endophyte infection levels were determined on three occasions by harvesting 30 tillers from each plot and checking their endophyte status. At the first assessment in November 1997, epidermal sheath strips were taken from each pseudostem and stained in aniline blue before they were checked for the presence of endophyte under the microscope. For the samples taken in April 1998 and February 1999, the endophyte status of each tiller was determined using a modification of the polyclonal antibody immunoblot procedure of Gwinn et al. (1991) in which a goat anti-rabbit conjugate was substituted for protein-A conjugate.

Argentine stem weevil damage was assessed on 20–25 tillers taken randomly from each plot on 18 February 1998 and 25 tillers sampled on 16 February 1999. Tillers were cut at ground level and were refrigerated until assessment within a fortnight of sampling. At assessment, the leaf lamina of each tiller was scored for adult feeding on a scale of 0–5, where 0 = no feeding and 5 = feeding scars all over the leaf. Pseudostems were stripped and examined for the presence of eggs and larval damage. Larval damage was categorised as minor if only a small entry/exit hole was present and the tiller was not

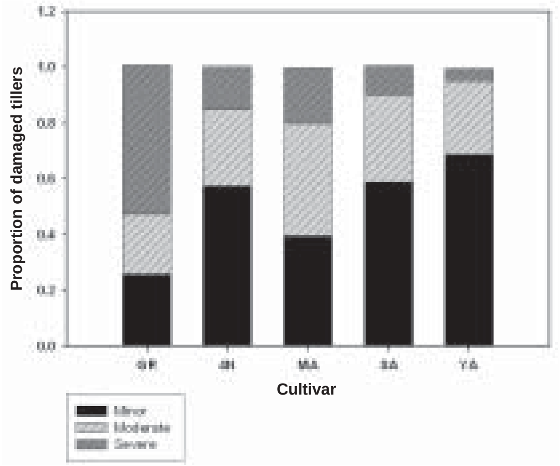

Fig. 1 Predicted mean proportion of damaged tillers classed as having minor, moderate or severe damage in five ryegrass cultivars, 'Greenstone' (GR), '4N', 'Marsden' (MA), 'Samson' (SA), and 'Yatsyn' (YA), in 1999.

visibly affected by the damage, moderate if some mining of the tiller had taken place, or severe if the tiller had been extensively mined. In the moderate and severe categories, the emergent leaf lamina was often wilting and yellow and the tiller was unlikely to survive.

Alkaloid levels were determined by HPLC in ryegrass (a) leaf lamina and (b) pseudostem (leaf sheath and emerging lamina) and flowering stem and seedhead when present, taken from 20 samples per plot at what was the estimated grazing height. Samples were taken during mid September/early October 1997, January and April 1998, and January 1999 during the 18-day grazing periods when grasses were being assessed for milk solids production by dairy cows.

Statistical analysis

Differences between cultivars in the percent endophyte infection levels, adult feeding scores, egg numbers, and percent larval damage were determined by analysis of variance. The damage severity data were analysed using a generalised linear model with binomial errors and a logit link in Genstat to give predicted mean proportions of damaged tillers with minor, moderate or severe damage. Alkaloid concentrations were compared in a generalised linear model using percent endophyte infection levels as a covariate.

Relationships between ASW feeding (adult feeding score and larval damage), endophyte infection rates, and alkaloid concentrations were investigated initially by univariate regression. Since alkaloid concentration was almost always linearly correlated with endophyte infection levels, bivariate and best subsets regressions were also used to estimate the relative contribution of each factor to ASW damage. A two-way ANOVA was carried out using alkaloids and endophyte percentage as covariates to determine the effects of ploidy and species on ASW adult feeding and larval damage, and on alkaloid content in leaf lamina and pseudostem. These analyses were only carried out on 1999 data when endophyte levels in the different treatments were uniformly high.

RESULTS

Endophyte infection rates

In November 1997, 8 months after sowing, the overall endophyte infection rate in the trial was 74%, with an average level of 61% in 'Greenstone', 70% in 'Marsden', 71% in '4N', 85% in 'Yatsyn' and 86% in 'Samson'. The endophyte level in

Table 1 Adult Argentine stem weevil feeding score, number of eggs and percentage of tillers with larval damage recorded on five ryegrass cultivars in February 1998 and 1999.

	1998			1999		
Cultivar	Adult feeding score	No. eggs/tiller	% tillers with larval damage	Adult feeding score	No. eggs/tiller	% tillers with larval damage
'Greenstone'	1.56	0.08	29.4	2.07	0.04	42.2
'4N'	0.96	0.10	13.5	1.49	0.08	30.2
'Marsden'	0.66	0.03	8.9	1.26	0.04	16.9
'Samson'	0.56	0.02	5.9	1.20	0.03	23.5
'Yatsyn'	0.50	0.07	10.6	1.03	0.18	18.2
SEM	0.44	0.11	9.1	0.29	0.09	7.78

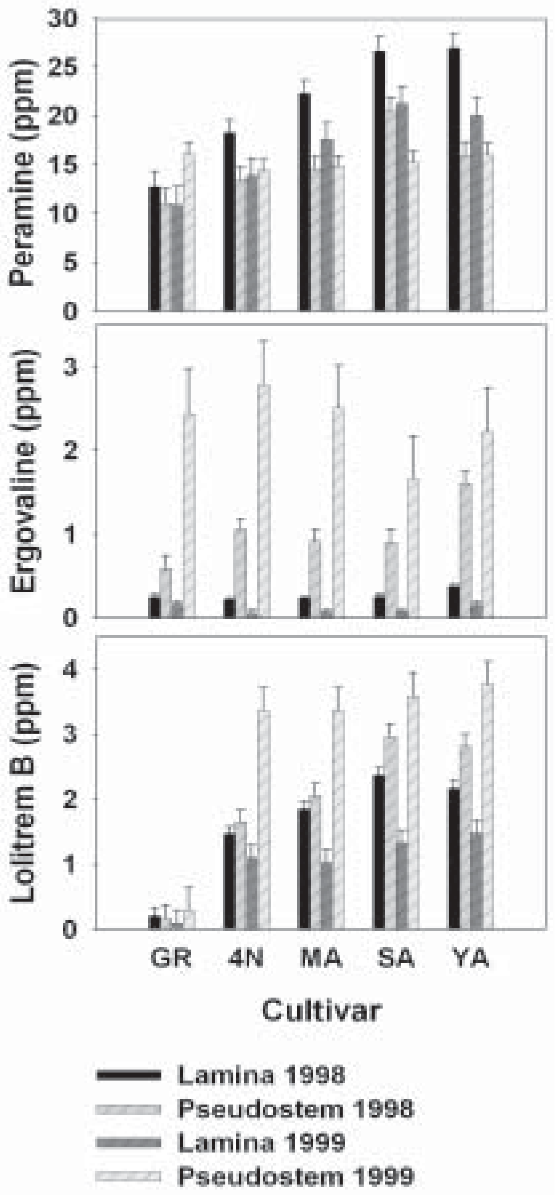

Fig. 2 Mean concentrations, calculated using percent endophyte as a covariate, of **A**, peramine; **B**, ergovaline; and **C**, lolitrem B in leaf lamina and pseudostem of five cultivars sampled in January 1998 and 1999. Error bars are SEMs for comparisons between cultivars for each plant part and sampling time. Abbreviations as for Fig. 1.

'Greenstone' was significantly lower than that in all other cultivars except 'Marsden'. By April 1998, the frequency of infection had increased substantially in all cultivars (mean 95%) with 'Greenstone' showing the largest increase, reaching an infection rate of 88%. Endophyte levels continued to increase and, by February 1999, the mean infection level in 'Greenstone' plots was 94%, with a mean for all cultivars of 97%.

Argentine stem weevil damage

Adult ASW feeding score was higher on 'Greenstone' than on all other cultivars ($P < 0.01$) in both years and also greater on '4N' relative to the other three cultivars, although this difference was significant only for 'Yatsyn' in 1998 and 'Samson' and 'Yatsyn' in 1999 (Table 1). 'Marsden', 'Samson' and 'Yatsyn' had similar feeding scores in both years. Few eggs were found on any treatment in 1998 or 1999, with no differences between treatments.

Larval damage to the different cultivars followed a very similar pattern in both years to the adult feeding scores (Table 1). The percentage of 'Greenstone' tillers damaged by larvae was substantially higher ($P < 0.01$) than for all other cultivars, while '4N' sustained more larval damage than 'Marsden', 'Samson', and 'Yatsyn'. The difference between '4N' and these three cultivars was not significant in 1998, but was in 1999. There were no differences between 'Marsden', 'Samson', and 'Yatsyn'.

Of the tillers damaged by larvae in 1998, the predicted mean proportion categorised as having minor damage was higher for 'Marsden' and 'Yatsyn' than for '4N', 'Greenstone', and 'Samson' (data not presented). As a result, the three latter cultivars tended to have higher proportions of damaged tillers with moderate or severe damage, but the differences in these categories were not significant. In 1999, most of the damage to tillers of '4N', 'Samson', and 'Yatsyn' was classed as minor, whereas 'Greenstone' had fewer tillers with minor damage and more with severe damage ($P < 0.05$) (Fig. 1). 'Marsden' also had fewer tillers with minor damage than '4N', 'Samson' and 'Yatsyn' ($P < 0.05$), but had more rated as having moderate damage although the differences were not significant in this category. Marsden had a considerably lower proportion of severely damaged tillers (20%) than did Greenstone (53%), but a higher proportion than Yatsyn (5%).

Alkaloids

Data are presented only for the samples taken for alkaloid analysis in January 1998 and 1999, and not for the samples taken in September 1997 and April 1998.

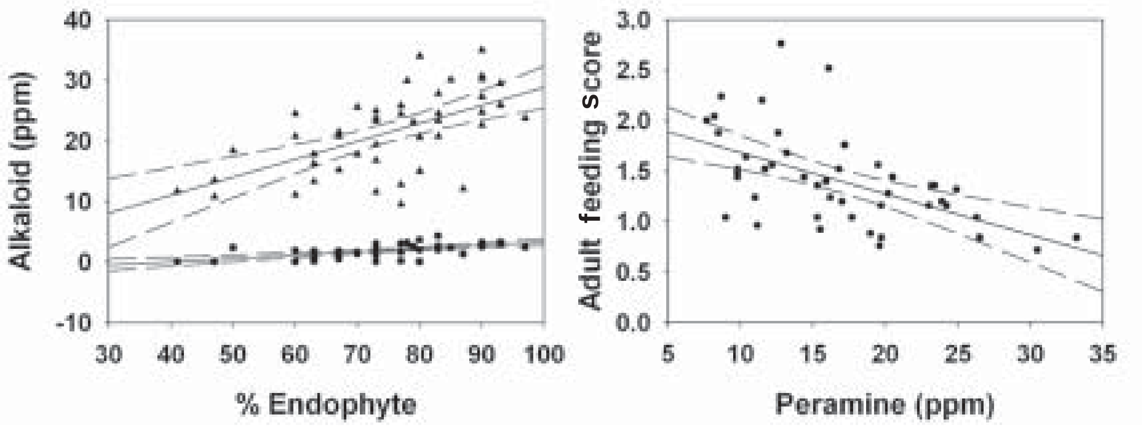

Fig. 3 Relationships between **A**, percent endophyte and the alkaloids, peramine (▲) and lolitrem B (●) in 1998, and **B**, peramine concentration in the leaf lamina and adult Argentine stem weevil feeding score in 1999, with 95% confidence intervals.

The relative amounts of peramine present in the leaf lamina of the different cultivars were the same in January of both years (Fig. 2A). Concentrations were lowest in 'Greenstone', highest in the diploids, 'Samson' and 'Yatsyn' and intermediate in '4N' and 'Marsden'. In 'Greenstone', leaf lamina peramine was significantly lower than that in other cultivars in 1998 and lower than that in 'Samson' and 'Yatsyn' in 1999. Leaf peramine content in '4N' was also lower than in 'Samson' and 'Yatsyn', while levels in 'Marsden' were not significantly different from these three cultivars. In 1998, peramine levels in the pseudostem were higher in 'Samson' than in 'Marsden', 'Greenstone' and '4N', but similar to 'Yatsyn'. In the following year, pseudostem peramine was similar across all cultivars (Fig. 2A).

Leaf lamina ergovaline in 'Yatsyn' was greater than in all other cultivars, and in the pseudostem was greater than in 'Greenstone', 'Marsden', and 'Samson' in 1998 (Fig. 2B). In 1999, leaf lamina ergovaline in 'Greenstone' was higher than in '4N' ($P < 0.05$), while all cultivars had similar levels in the pseudostem. Low levels of lolitrem B occurred in 'Greenstone', indicating some contamination of these plots with wild-type endophyte (Fig. 2C). Of the other cultivars, leaf lamina and pseudostem levels of lolitrem B were lower in '4N' than in 'Samson' and 'Yatsyn' in 1998, but no significant differences were found in 1999.

Relationship between endophyte, alkaloids, and Argentine stem weevil damage

In 1998, levels of lolitrem B and peramine in leaf lamina and pseudostem were linearly correlated with percent endophyte within each sward (Fig. 3A), whereas the relationship between ergovaline and percent endophyte was not significant (data not shown). ASW adult feeding was negatively correlated with percent endophyte (data not shown), with concentrations of peramine in the leaf lamina (Fig. 3B) and with both lolitrem B and ergovaline (data not shown). Bivariate regressions for ASW

Table 2 Probabilities in bivariate regressions determining relative importance of percent endophyte and concentration of each alkaloid in leaf lamina on Argentine stem weevil adult feeding score or in the pseudostem on proportion of tillers with larval damage.

	Year	% endophyte	Peramine	% endophyte	Lolitrem B	% endophyte	Ergovaline
Adult feeding	1998	0.181	0.059	0.718	0.002	0.018	0.104
	1999	0.059	<0.001	0.099	<0.001	0.020	0.911
Larval damage	1998	0.680	0.142	0.134	<0.001	0.318	0.168
	1999	0.015	0.163	0.361	0.001	0.013	0.215

adult feeding score that combined percent endophyte with each alkaloid as predictors were also highly significant ($P < 0.01$). Partial significance relating to each factor indicated that leaf lolitrem B concentration was a major determinant of adult feeding score, although leaf peramine also came close to significance (Table 2). Graphical representation indicated that 'Greenstone', which does not produce lolitrem B, was having a major influence on the outcome of this regression and this was confirmed when, after excluding these data, the correlation between adult feeding and lolitrem B was no longer significant. Best subsets regression gave the highest correlation with the inclusion of all three alkaloids (43.3%) or percent endophyte and alkaloid concentration (42%).

As for 1998, ASW adult feeding was again correlated with percent endophyte in 1999 (P = 0.019) and also individually with levels of peramine and lolitrem B ($P < 0.001$) in the leaf, but not with ergovaline. Unlike 1998, however, alkaloid concentrations were not correlated with percent endophyte. In the bivariate regressions, peramine and lolitrem B, rather than percent endophyte, were the significant contributors to adult feeding score (Table 2). The absence of lolitrem B in 'Greenstone' was again a dominant factor in the significant correlation between adult feeding and this alkaloid, with the relationship no longer significant after removal of these data. In contrast to this, the regression between adult feeding and leaf lamina peramine remained significant even after exclusion of the 'Greenstone' data. With best subsets regression the highest correlation coefficient was obtained using all factors (38.3%) or using percent endophyte, peramine, and ergovaline (37.8%).

In both years, the percentage of tillers damaged by larvae was linearly related to adult feeding (1998: $y = 1.51 + 14.3x$, $P < 0.001$, $R^2 = 45.5\%$; 1999: $y = -1.92 + 20.0x$, $P < 0.001$, $R^2 = 58.6\%$). In bivariate regressions, with percent endophyte and each alkaloid, only pseudostem lolitrem B was a significant predictor of larval damage in both years (Table 2). In 1998, a combination of adult feeding score, percent endophyte and pseudostem lolitrem B was the best predictor of larval damage, accounting for 53.6% of the variation. In 1999, the highest correlation coefficient of 66.7% was achieved using all three alkaloids and adult feeding score, but this was only marginally higher than when ergovaline was not included.

Effects of ploidy and species

Leaf lamina concentration of peramine was higher in diploid and perennial cultivars than in the tetraploids and hybrids respectively, using percent endophyte as a covariate in the analysis (Table 3). Peramine in the pseudostem, and ergovaline levels in lamina and pseudostem, were not affected by ploidy or species. Lolitrem B concentration was not analysed due to the absence of this alkaloid in 'Greenstone'.

ASW adult feeding was significantly higher in tetraploid and hybrid than in diploid and perennial cultivars, respectively, when percent endophyte only was used as the covariate, but when it included leaf

Table 3 Effect of ploidy and species on mean alkaloid concentration and Argentine stem weevil adult feeding score and log percentage of tillers with larval damage. E, endophyte; Per-L, leaf lamina peramine concentration; Per-S, pseudostem peramine; Erg-S, pseudostem ergovaline; Lol-S, pseudostem lolitrem B.*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

		Ploidy		Species	
Variate	Covariate	Diploid	Tetraploid	Hybrid	Perennial
Peramine-L	% E	19.73	12.23***	14.31	18.34***
Ergovaline-L	% E	0.07	0.11 NS	0.11	0.07 NS
Peramine-S	% E	15.00	15.20 NS	15.23	14.95 NS
Ergovaline-S	% E	2.05	2.59 NS	2.39	2.25 NS
Adult feeding	% E	1.2	1.8***	1.6	1.3**
Adult feeding	% E, Per-L	1.3	1.6 NS	1.5	1.4 NS
% larval damage	% E	20.2	36.3***	29.0	27.4 NS
% larval damage	%E, Per-S, Erg-S, Lol-S	20.7	33.6**	24.6	26.7 NS

lamina peramine the significant effect was eliminated (Table 3). A significant ploidy by species interaction ($P < 0.05$) occurred when using percent endophyte alone, or with peramine, as a covariate, such that ASW adult feeding on the tetraploid hybrid cultivar ('Greenstone') was higher than that on all other combinations. The tetraploid cultivars also sustained more larval damage than the diploids, but there was no significant species effect whether alkaloids were included as covariates or not (Table 3).

DISCUSSION

In the absence of endophyte, hybrid and annual ryegrasses are more susceptible than perennials to ASW damage (Barker 1989; Prestidge 1991). Goldson (1982) postulated that lower cellulose levels in the Italian and hybrid ryegrasses increased their vulnerability to ASW attack, while Barker (1989) demonstrated an association between ASW feeding intensity and lower fibre content (hemicellulose, cellulose, and lignin) in the leaf blades of *L. perenne* and *L. multiflorum*. Feeding by adult weevils is correlated with oviposition in the sheath (Kain et al. 1982; Barker 1989), but this does not account for all the variation. Plant factors such as increased uptake and deposition of silica in leaf sheaths are negatively correlated with oviposition and are another reason for the higher susceptibility of *L. multiflorum* cultivars to ASW damage (Barker 1989). When a ryegrass is also infected by *Neotyphodium* endophyte, the acceptability of a particular plant as a host of ASW must be a balance between attractive components of the plant such as reduced fibre and silica content and the deterrence exerted by the endophyte. Further to these aspects, both plant host and endophyte strain and interactions between these factors can modify the quantity and type of alkaloids produced (Latch 1994; Ball et al. 1995; Easton et al. 2002). The possibility of synergy between alkaloids influencing the insect response should also be considered. Thus, the number of factors which can influence ASW are both varied and complex.

In results presented here, both ploidy and species have affected the amount of ASW damage. Of the five cultivars in this trial, the three diploids, 'Yatsyn', 'Samson', and 'Marsden', had similar and relatively low levels of ASW damage compared with the tetraploid/hybrid cultivar, 'Greenstone'. Intermediate between these was the tetraploid/perennial, '4N'. The reasons for these differences, however, are difficult to determine. Plant host, endophyte infection levels within a pasture, and peramine concentration all, individually, are elements influencing ASW, but interactions between them have also occurred. Interpretation of the results has been further complicated by the presence of an endophyte strain in 'Greenstone' which does not produce lolitrem B and, initially, relatively low endophyte infection rates in this cultivar and in 'Marsden'.

The frequency of endophyte infection in ryegrass tillers in pasture is a major determinant of ASW damage (Barker et al. 1989; Barker & Addison 1993; Popay et al. 1999), and was a major factor in the first season of the trial reported here. The selective advantage of having endophyte is also a well recognised phenomenon resulting in increasing levels of endophyte in a sward over time (e.g., Prestidge et al. 1984, 1985; Popay et al. 1999), and was apparent in this trial between the two years. Peramine is a powerful feeding deterrent to adult ASW (Rowan et al. 1990) and is thus the major metabolite produced by *Neotyphodium* endophytes that influences ASW behaviour toward individual plants. Maintaining a high peramine concentration, estimated to be a minimum of between 15 and 20 ppm (Popay & Wyatt 1995), is therefore crucial for strong resistance to ASW. Leaf lamina concentrations of peramine were less than 15 ppm in 'Greenstone' in both years and in '4N' in the 1999 season. In both years, regression analysis also indicated that lolitrem B was a factor affecting adult feeding. Clearly, the absence of this alkaloid in 'Greenstone' had a major effect on the analyses and produced relationships that seem not to be biologically valid since lolitrem B, by itself, does not affect adult feeding (Prestidge & Gallagher 1985). Nevertheless, a synergistic interaction between peramine and lolitrem B which strengthens ASW avoidance of those cultivars containing endophytes that produce lolitrem B cannot be ruled out. Adult ASW are also responsive to ergovaline (Popay et al. 1991), but this has not had an important influence because of the low concentrations of this alkaloid in the leaf lamina.

Overall leaf peramine concentration was significantly affected by both ploidy and species and was correlated with ASW adult feeding. In the covariate analysis, adult feeding score was significantly affected by ploidy and species when endophyte percentage alone was used as the covariate but not when peramine leaf concentration was included. This suggests that ploidy and species are only affecting adult ASW behaviour indirectly via the peramine concentration. However, with both peramine and percent endophyte as covariates, a significant ploidy × species interaction still occurred, with higher adult feeding on the tetraploid/hybrid combination ('Greenstone') than on all other combinations, indicating that peramine was not the only factor affecting adult behaviour in this cultivar. Furthermore, best subsets regressions with combinations of percent endophyte and alkaloid content could only account in most cases for less than 50% of the variation in adult feeding. The attractiveness factors which are responsible for the greater susceptibility of the tetraploid and hybrid cultivars are therefore also likely to have had a role in adult ASW activity.

Tiller damage by ASW larvae is largely a function of oviposition which in turn is a function of adult feeding intensity. Due to the time of sampling, few eggs were found in any treatment, but a significant relationship was demonstrated between adult feeding and larval damage. Thus, the factors such as ploidy, species and endophyte that determine adult feeding will also be major determinants of the number of tillers damaged by larvae. Nevertheless, endophyte can provide a secondary line of defence against larvae, through the deterrency of peramine (Rowan et al. 1990) and the effects of lolitrem B on growth and development (Prestidge & Gallagher 1985), reducing both the number of tillers damaged by larvae and the severity of that damage. Given the strong association between adult feeding and larval damage, the absence of lolitrem B in 'Greenstone' was probably not a major factor in the number of tillers with larval damage. A similar conclusion was reached in other trials with endophytes that are deficient in this metabolite (Popay et al. 1995, 1999). However, 'Greenstone' not only had more damaged tillers than other treatments but also had the highest proportion of damaged tillers classed as being severely affected. Since peramine levels in the pseudostem did not differ between treatments, it seems likely that the absence of lolitrem B in this cultivar was responsible for the severity of the damage it sustained.

Despite the less robust protection against ASW provided by endophyte in the tetraploid/hybrid cultivars compared with that in perennials, damage by ASW is considerably lower in endophyte-infected plants than in endophyte-free (Piggot et al. 1988; Prestidge 1991; Popay et al. 1995). The critical difference between the perennial and tetraploid/hybrid cultivars appears to be the concentration of peramine in the leaf lamina. Typically in perennial ryegrass cultivars, peramine concentrations are higher in leaf lamina than in leaf pseudostems (Ball et al. 1995; Keogh et al. 1996). Our results show a similar distribution of peramine in the perennial cultivars, but in the hybrid/tetraploid cultivars the amount of peramine in the leaf lamina is very similar to, or occasionally less than, the amount in the pseudostem. In individual plants, yearly mean concentration of alkaloids, such as peramine and lolitrem B, are closely related to mean concentration of *N. lolii* (Ball et al. 1995), although this relationship does not hold true for distribution of peramine and mycelia within specific plant parts (Keogh et al. 1996). Unlike leaf lamina peramine, ergovaline and lolitrem B in lamina and pseudostems, and peramine in the latter are not affected by ploidy or species. Thus, the reasons for the relatively low concentration of peramine in the upper parts of the tetraploid/hybrid plants are unlikely to be due to a lower endophyte concentration, but may be due to other factors related to the plant/endophyte interaction.

ACKNOWLEDGMENTS

We thank Neil Cox and David Baird for statistical analysis and Liz Davies and Joanne Baltus for technical assistance.

REFERENCES

Ball, O. J-P.; Prestidge, R. A.; Sprosen, J. M. 1995: Interrelationships between *Acremonium lolii*, peramine and lolitrem B in perennial ryegrass. *Applied and Environmental Microbiology 61*: 1527–1533.

Barclay, P. C. 1963: Ariki, a new selected perennial-type ryegrass variety. *Proceedings of the New Zealand Grassland Association 25*: 128–137.

Barker, G. M. 1989: Grass host preferences of *Listronotus bonariensis* (Coleoptera: Curculionidae). *Journal of Economic Entomology 82*: 1807–1816.

Barker, G. M.; Addison, P. J. 1993: Argentine stem weevil populations and damage in ryegrass swards of contrasting *Acremonium* infection. Proceedings of the 6th Australasian Grassland Invertebrate Ecology Conference. Pp. 161–168.

Barker, G. M.; Pottinger R. P.; Addison, P. J. 1989: Population dynamics of the Argentine stem weevil (*Listronotus bonariensis*) in pastures of Waikato, New Zealand. *Agriculture, Ecosystems and Environment 26*: 79–115.

Easton, H. S.; Latch, G. C. M.; Tapper, B. A.; Ball, O. J-P. 2002: Ryegrass host genetic control of concentrations of endophyte derived alkaloids. *Crop Science 42*: 51–57.

Goldson, S. L. 1982: An examination of the relationship between Argentine stem weevil (*Listronotus bonariensis* (Kuschel)) and several of its host grasses. *New Zealand Journal of Agricultural Research 25*: 395–403.

Gwinn, K. D.; Collins-Shepard, M. H.; Reddick, B. B. 1991: Tissue print-immunoblot, an accurate method for the detection of *Acremonium coenophialum* in tall fescue. *Phytopathology 81*: 747–748.

Hoy, J. M. 1960: Argentine stem weevil damage to ryegrass in the North Island. *New Zealand Journal of Agriculture 100*: 78–81.

Kain, W. M.; Wyeth, T. K.; Gaynor, D. L.; Slay, M. W. 1982: Argentine stem weevil (*Hyperodes bonariensis* Kuschel) resistance in perennial and hybrid ryegrasses. 1. Field resistance in perennial ryegrass (*Lolium perenne* L.). *New Zealand Journal of Agricultural Research 25*: 255–259.

Kelsey, J. M. 1958: Damage in ryegrass by *Hyperodes griseus* Hust. *New Zealand Journal of Agricultural Research 1*: 790–795.

Keogh, R. G.; Tapper, B. A.; Fletcher, L. H. 1996: Distributions of the fungal endophyte *Acremonium lolii*, and of the alkaloids lolitrem B and peramine, within perennial ryegrass. *New Zealand Journal of Agricultural Research 39*: 121–127.

Latch, G. C. M. 1994: Influence of *Acremonium* endophytes on perennial grass improvement. *New Zealand Journal of Agricultural Research 37*: 311–318.

Piggott, G. J.; Morgan, H. M.; Bakker, R. J. 1988: Endophytes in Northland ecotypes of hybrid ryegrass. Proceedings of the 41st New Zealand Weed and Pest Control Conference. Pp. 138–140.

Popay, A. J.; Wyatt, R. T. 1995: Resistance to Argentine stem weevil in perennial ryegrass infected with endophytes producing different alkaloids. Proceedings of the 48th New Zealand Plant Protection Conference. Pp. 229–236.

Popay, A. J.; Prestidge, R. A.; Rowan, D. D.; Dymock, J. J. 1991: The role of *Acremonium lolii* mycotoxins in insect resistance of perennial ryegrass (*Lolium perenne*). *In*: Quisenberry, S. S.; Joost, R. E. *ed*. Proceedings of the International Symposium *Acremonium*/Grass Interactions. Pp. 44–46.

Popay, A. J.; Hume, D. E.; Mainland, R. A.; Saunders, C. J. 1995: Field resistance to Argentine stem weevil (*Listronotus bonariensis*) in different ryegrass cultivars infected with an endophyte deficient in lolitrem B. *New Zealand Journal of Agricultural Research 38*: 519–528.

Popay, A. J.; Hume, D. E.; Baltus, J. G.; Latch, G. C. M.; Tapper, B. A.; Lyons, T. B.; Cooper, B. M.; Pennell, C. G.; Eerens, J. P. J.; Marshall, S. L. 1999: Field performance of perennial ryegrass (*Lolium perenne*) infected with toxin-free fungal endophytes (*Neotyphodium* spp.). *In*: Ryegrass endophyte: An essential New Zealand symbiosis. New Zealand Grassland Association. *Grassland Research and Practice Series No. 7*: 113–122.

Prestidge, R. A. 1991: Susceptibility of Italian ryegrass (*Lolium multiflorum* Lam) to Argentine stem weevil (*Listronotus bonariensis* (Kuschel)) feeding and oviposition. *New Zealand Journal of Agricultural Research 34*: 119–125.

Prestidge, R. A.; Gallagher, R. T. 1985: Lolitrem B – a stem weevil toxin isolated from *Acremonium*-infected ryegrass. Proceedings of the 38th New Zealand Weed and Pest Control Conference. Pp. 38–40.

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. Pp. 119–122.

Prestidge, R. A.; van der Zijpp, S.; Badan, D. 1984: Effects of Argentine stem weevil on pastures in the Central Volcanic Plateau. *New Zealand Journal of Experimental Agriculture 12*: 323–331.

Prestidge, R. A.; di Menna, M. E.; van der Zjipp, S.; Badan, D. 1985: Ryegrass content, *Acremonium* endophyte and Argentine stem weevil in pastures in the volcanic plateau. Proceedings of the 38th New Zealand Weed and Pest Control Conference. Pp. 41–44.

Rowan, D. D.; Dymock, J. J.; Brimble, M. A. 1990: Effect of fungal metabolite peramine and analogs on feeding and development of Argentine stem weevil (*Listronotus bonariensis*). *Journal of Chemical Ecology 16*: 1683–1695.

Timlin, J. S. 1964: Diagnosis and extent of damage by the stem weevil. Proceedings of the 17th New Zealand Weed and Pest Control Conference. Pp. 149–151.

Whatman, C. P. 1959: Damage to pastures by wheat stem weevil. *New Zealand Journal of Agriculture 98*: 551–552.