

Occurrence and impact of pasture mealybug (*Balanococcus poae*) and root aphid (*Aploneura lentisci*) on ryegrass (*Lolium* spp.) with and without infection by *Neotyphodium* fungal endophytes

C. G. L. PENNELL¹

A. J. POPAY^{2*}

O. J-P. BALL^{3†}

D. E. HUME³

D. B. BAIRD¹

¹AgResearch Lincoln
Private Bag 60
Lincoln, New Zealand

²AgResearch Ruakura
Private Bag 3123
Hamilton, New Zealand

³AgResearch Grasslands
Private Bag 11 008
Palmerston North, New Zealand

*Author for correspondence.

†Present address: Northland Polytechnic, Private Bag 9019, Whangarei, New Zealand.

Abstract Pasture mealybug (*Balanococcus poae*) was found infesting two field trials evaluating the performance of selected strains of the endophyte *Neotyphodium lolii* in ryegrass (*Lolium* spp.) in Canterbury, New Zealand. Deterioration of endophyte-free plots relative to endophyte-infected plots had been observed. In Trial A, pasture mealybug were sampled in plots of the perennial ryegrass cultivar 'Grasslands Nui', without endophyte (nil), or infected with the wild-type endophyte, or the selected strains, AR1 and AR37. In Trial B, mealybug numbers on six ryegrass cultivars infected with AR1 or wild-type were compared with those on the same cultivars without endophyte. In sampling these trials, the presence of a root aphid, *Aploneura lentisci*, was also noted. Populations of mealybug in both trials were similar on all endophyte-infected treatments and significantly lower than populations on nil treatments. Neither AR1 nor wild-type appeared

to reduce root aphid numbers, while AR37 may have had some effect. The amount of dead grass was significantly greater in nil than in endophyte-infected plots in Trial A, and yield of ryegrass was correlated with numbers of mealybug and root aphid present. Pasture productivity in nil treatments had recovered by spring, in the year of the outbreak. The presence of endophyte, strain of endophyte and cultivar were all significant factors affecting both total dry matter and green yield in Trial B. Pasture mealybug accounted for 55% of the variation in a decline in growth rate that occurred in this trial over the summer-autumn periods between 2000 and 2001, particularly in the nil treatments. Two years after the outbreak there was 25% less ryegrass and persistently lower pasture yields in nil plots. We conclude that pasture mealybug are capable of inflicting severe damage to endophyte-free ryegrass in Canterbury, particularly during dry summer-autumn periods.

Keywords endophyte; plant resistance; *Neotyphodium lolii*; perennial ryegrass; *Balanococcus poae*; *Aploneura lentisci*; drought; pasture production

INTRODUCTION

Mealybugs are small insects that suck the sap from plant tissues, causing damage to a wide range of host plants. Several species infest grasses, where they may be found under the leaf sheath, in nodes, in the crown or on roots. One such species, pasture mealybug (*Balanococcus poae* (Maskell) Hemiptera: Pseudococcidae), is endemic to New Zealand where it is known to occur in the Canterbury, Nelson, and Manawatu regions (Pearson 1988) on a range of endemic and introduced graminoids as well as *Carex* spp., *Gaultheria depressa* Hook. f., and *Acaena* spp. (de Boer 1968; Cox 1987). Crawlers are dispersed by wind, while older nymphs and adults become sedentary, surrounding themselves with white, flocculent wax secretions once they become established on crowns and upper roots of host grasses. Although they were originally thought to be

entirely parthenogenic and to have one generation a year, there is now evidence of sexual reproduction and of two generations during the spring-autumn period (Pennell et al. 2004).

The root aphid (*Aploneura lentisci* (Pass.) Hemiptera: Aphididae) is commonly found throughout New Zealand on roots of various Graminae and on some weeds such as *Ranunculus acer* (Cottier 1953). Mature root aphid are pale yellow to cream coloured and generally smaller than mature pasture mealybug, but also produce white flocculent wax as they feed on phloem. Colonies of root aphid may develop anywhere in the root system from the crown to deep in the soil profile.

Pearson (1988) reported that the presence of the fungal endophyte *Neotyphodium lolii* (Latch, Christensen, and Samuels) Glenn, Bacon, and Hanlin, protected its host ryegrass from pasture mealybug in pot trials. This was the wild-type endophyte that occurs naturally in perennial ryegrass (*Lolium perenne* L.) throughout New Zealand. It produces three known alkaloids: peramine, lolitrem B, and ergovaline, and is important to the New Zealand agricultural industry for two contrasting reasons. Firstly, livestock feeding on infected grass may suffer health problems caused by lolitrem B and ergovaline, including ryegrass staggers and heat stress (Fletcher et al. 1999). Secondly, ryegrass plants infected with *N. lolii* are resistant to attack from several insects, including Argentine stem weevil (*Listronotus bonariensis* (Kuschel) which is deterred by the presence of the alkaloid peramine (Rowan et al. 1990). Research has focused on finding naturally-occurring endophyte strains with the potential to eliminate the adverse mammalian effects while retaining the insect biocontrol component of *N. lolii* infection. This has resulted in the recent release of ryegrass cultivars infected with AR1, an endophyte sourced originally from Europe, which provides resistance to Argentine stem weevil (Popay et al. 1999) but is not toxic to livestock (Fletcher 1999). Other endophyte strains are also being trialled for potential use in New Zealand pastures, including the AR37 strain. This strain produces none of the known alkaloids, peramine, ergovaline or lolitrem B but, despite this, gives resistance to Argentine stem weevil (Popay & Wyatt 1995).

In autumn 1998, a rapid deterioration of endophyte-free ryegrass plots compared with endophyte-infected plots was observed in a trial at Lincoln, Canterbury, which was one of a series set up to evaluate the effect of various strains of endophytes on pasture productivity and insect damage (Popay

et al. 1999). On closer examination, some ryegrass plants were found to be infested with pasture mealybug. A similar infestation of mealybug occurred in a trial evaluating the performance of AR1 in various cultivars in autumn 2001. When sampling these trials for mealybug, the root aphid, *A. lentisci*, was also found to be present. Their occurrence in these trials provided an opportunity to determine the effects of various endophyte strains on populations of these species. In addition, by using available yield and point analysis data, we were also able to examine the impact of these insects on pasture productivity.

MATERIALS AND METHODS

Mealybug and root aphid numbers were determined on two dryland trials (A and B) at Lincoln, Canterbury, New Zealand (mean annual rainfall 650 mm). Both trials were situated on a Wakanui silt loam of medium fertility, had no legume component and had nitrogen fertiliser applied in the form of urea or as calcium ammonium nitrate either after each grazing at a rate of 3% of the mean total herbage dry matter production of the three highest yielding treatments (Trial A, average of 300–350 kg/ha per year) or as 20 units applied twice in spring and twice in autumn (Trial B). Superphosphate at 200 kg/ha was applied annually. Rotational grazing by sheep maintained the sward composition without the need for herbicide applications. There were four replicates of each treatment arranged in a randomised block design. Endophyte infection levels were determined in autumn or late spring of each year by taking 25 (Trial A) or 20 (Trial B) tillers, cut at ground level, from each plot. Endophyte status of the tillers was determined by a tissue print immunoassay (Hahn et al. 2003).

Plots were assessed for dry matter (DM) using a calibrated rising plate meter and regression analysis from cut samples when DM reached about 2500–3000 kg/ha (Earle & McGowan 1979; Gourly & McGowan 1991). Green yield was estimated in Trial B by dissection of samples cut from three 0.6 m² quadrats in each plot. Ground cover was assessed using point analysis. A string line consisting of 50 points, 90 mm apart, running diagonally from one corner of each plot to the other was used. An observer walking directly above the string line scored the status of the pasture immediately below each point. Each point was scored for the presence of one of the following: live ryegrass, dead ryegrass or bare ground. Both diagonals in each plot were assessed

in this way with a total of 97 (Trial A) or 100 (Trial B) points per plot being scored. Point analysis was carried out on all treatments sampled for mealybug in Trial A in June 1998, and on all three endophyte treatments in the cultivars, 'Nui', 'Supreme', and 'Impact', in May 2003 in Trial B.

Trial A

This trial, evaluating the performance of different endophyte strains in perennial ryegrass cv. 'Grasslands Nui' (Popay et al. 1999), was sown in autumn 1996, in 5 × 3 m plots at a rate of 15 kg/ha viable seed. Four of the seven treatments in the trial, endophyte-free (nil) ryegrass and ryegrass infected with wild-type endophyte or two novel strains, AR1 and AR37, were sampled for pasture mealybug in June 1998. For sampling, 10, 50 mm diameter × 40 mm deep, cores were taken at random from each plot and stored in plastic bags at 4°C. The cores were then air dried for 1 h at 30°C in an air blast oven to reduce soil moisture and facilitate the breaking up of the soil. Mealybug and root aphid were removed by hand and their numbers recorded.

Trial B

This trial was a factorial of six ryegrass cultivars and three endophyte treatments. Three perennial ryegrass cultivars, 'Grasslands (G) Nui', 'G. Samson', and 'G. Pacific', and three long-rotation hybrid cultivars (*L. × boucheanum* syn. *L. hybridum*), 'G. Impact', 'G. Supreme', and 'G. Marsden' were all infected with either AR1 or wild-type endophytes or were endophyte-free. The trial was sown into 6 m² plots at a rate of 20 kg of viable seed/ha in autumn 1999. Mealybug and root aphid numbers were determined in late May 2001, by taking six soil cores (60 mm diameter to an approximate depth of 100 mm) per plot over grass plants. The soil in each core was thoroughly broken up and then wet sieved to obtain both insect species. The changes in sampling procedure from that used in Trial A were primarily aimed at improving estimations of root aphid populations. These insects occur at greater depths in the soil profile than mealybug and hence the soil samples were 100 mm rather than 40 mm deep. In addition, wet sieving allowed insect samples to be stored and counted later with the aid of a microscope, since many of the immature stages of root aphid in particular are not visible to the naked eye.

For counting, each sample was made up to a quantity of between 60 and 100 ml with tap water and stirred before removal of a 10 ml subsample as two 5 ml aliquots. Mealybug and root aphid were

counted in the subsample using a Petri dish with a grid marked on the base and with the aid of a stereo microscope (16× magnification).

Statistical analysis

Mealybug and root aphid numbers were analysed using an analysis of variance (ANOVA). After testing for normality and homogeneity of variances, log transformation was used to normalise the data in Trial A but was not necessary for Trial B. Arithmetic means are presented for both trials. Comparisons among endophyte treatments of dry matter yields and point analysis data were carried out using ANOVA. The impact of different parameters (endophyte, cultivar, and insect numbers) on pasture productivity was evaluated using covariate and regression analyses. In Trial B, the effects of mealybug on relative changes in pasture productivity of the different cultivar/endophyte treatments were determined by comparing growth rates over the summer/autumn period in 2001 (63 days from 19 March to 21 May) when mealybug was present, with the equivalent period the previous year (78 days from 29 January to 14 May) when no mealybug was apparent. An analysis of variance of the difference in growth rate during these periods was then carried out using proportion of endophyte infection and numbers of mealybug and root aphid as covariates to determine the relative roles of these factors.

RESULTS

Trial A

Endophyte infection rate, determined in November 1998, was 1% in nil plots, 89% in wild-type, and 98% in both AR1 and AR37 infected treatments.

Significantly more pasture mealybug were recovered from soil cores taken from nil plots compared with any of the three endophyte-infected treatments ($P < 0.01$) (Table 1). The numbers of mealybug were low in wild-type, AR1, and AR37 plots with no significant differences between these treatments. There were more root aphids on AR1 than on any of the other three endophyte treatments ($P < 0.05$), and more on wild-type than on AR37 ($P < 0.05$). The difference in root aphid numbers between AR37 and nil was not significant ($P = 0.278$).

There was more dead material and less live material ($P < 0.05$) in the nil treatment compared with the three endophyte-infected treatments (Table 1). The amount of live ryegrass present also differed

significantly ($P < 0.05$) among the three endophyte strains (AR1 < wild-type < AR37) (Table 1) and there was proportionately less dead ryegrass in AR37 plots than in AR1 ($P < 0.05$), with wild-type plots intermediate between these two treatments. The proportion of bare ground recorded was lower in AR37 plots than in nil and AR1 treatments ($P < 0.05$) but not significantly less than in wild-type. Yields of all ryegrass treatments were similar in the two measurements taken in October and November 1997. In February 1998, yield of ryegrass from the nil and AR1 treatments was significantly lower than the yield from the wild-type and AR37 treatments ($P < 0.01$) (Fig. 1). By May, pasture productivity of AR1 had recovered somewhat but was still lower than AR37 ($P < 0.05$) while nil yields remained significantly lower than those of the endophyte-infected treatments. Yields in the nil treatment had improved by August, with no significant differences recorded among treatments either then or in October.

Total yield in May was highly correlated with numbers of mealybug (a) and root aphid (b) ($y = 1754 - 39.4a - 41.1b$, $R^2 = 0.81$, $P < 0.0001$). The correlation coefficient was reduced to 0.67 if root aphid was not included in the regression. The changes in pasture productivity of each treatment between November 1997 and February 1998 and February and May 1998 (Fig. 1) could not be related statistically to density of mealybug in June. Root aphid were not analysed for their effect during these periods because they have a short lifecycle and numbers potentially could have changed considerably over that time.

Trial B

Endophyte infection levels in November 2000 were less than 10% in all nil cultivars except for 'Supreme' which had a 26% infection rate. All AR1 treatments were >90% infected with endophyte (mean = 96%) while frequency of infection in

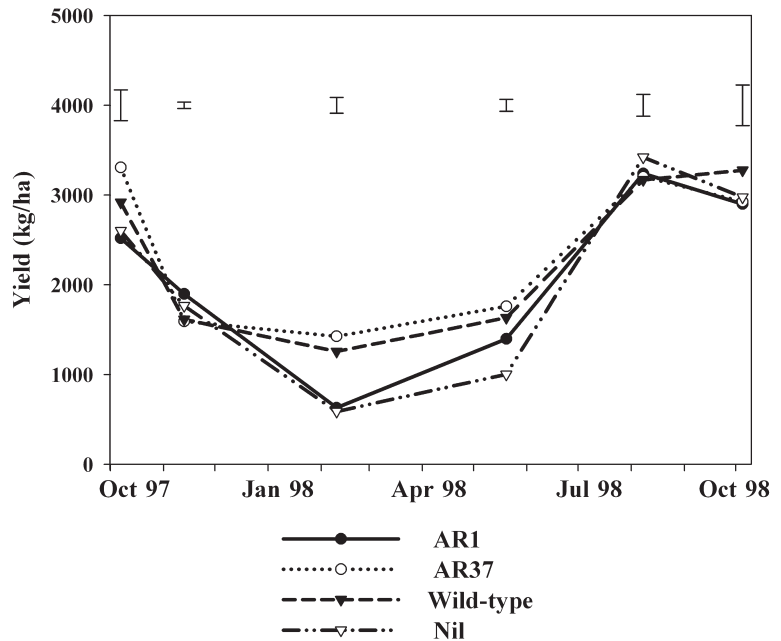
Table 1 Trial A: Mean numbers (and SE) of *Balanococcus poae* and *Aploneura lentisci* per core and percentage of live and dead grass or bare ground in perennial ryegrass cv. 'Grasslands Nui', without endophyte (nil) or infected with one of three strains of endophyte. Numbers in the same column with letters that differ from each other are significantly different at $P < 0.01$ for upper case letters and $P < 0.05$ for lower case letters.

Treatment	<i>B. poae</i> (no. per core)	<i>A. lentisci</i> (no. per core)	Live grass (%)	Dead grass (%)	Bare ground (%)
Nil	16.8 ^A (2.2)	2.1 ^{ab} (0.9)	37.1 (1.3)	31.2 (1.5)	31.7 (1.5)
Wild-type	0.6 ^B (0.2)	2.9 ^b (0.9)	61.8 (2.5)	12.9 (2.0)	25.3 (1.2)
AR1	1.0 ^B (0.3)	7.0 ^c (1.0)	49.0 (3.1)	19.3 (5.1)	31.7 (2.9)
AR37	0.3 ^B (0.2)	0.3 ^a (0.2)	71.7 (4.0)	8.5 (2.7)	19.8 (3.4)
LSD (5%)			8.56	9.14	8.83

Table 2 Trial B: Mean number of *Balanococcus poae* and *Aploneura lentisci* per core in six different ryegrass cultivars, without endophyte (nil) or infected with wild-type or AR1 endophytes. NS = not significant.

Cultivar	<i>B. poae</i> (no. per core)			<i>A. lentisci</i> (no. per core)			
	Nil	Wild-type	AR1	Nil	Wild-type	AR1	
'Pacific'	64	21	7	101	139	215	
'Impact'	72	1	2	203	128	145	
'Nui'	125	2	14	106	70	142	
'Samson'	155	10	3	158	174	115	
'Marsden'	52	10	5	102	102	182	
'Supreme'	43	10	6	130	102	93	
Mean	84	9	6	133	119	149	
5% LSD (d.f.)		Cultivar 32.6 (15) Endophyte 19.2 (36) Cultivar × Endophyte 48.9 (51)			Cultivar NS Endophyte NS Cultivar × Endophyte 79.5 (51)		

Fig. 1 Mean yields (and SED) between 7 October 1997 and 5 October 1998 of perennial ryegrass cv. 'Grasslands Nui' without endophyte or infected with the endophyte strains, AR1, wild-type or AR37.



wild-type treatments ranged from 66% in 'Supreme' to 91% in 'Impact' (mean = 82%). These infection levels remained stable over the next 2 years for most treatments, increasing by less than 10%. The most notable exceptions were infection levels in endophyte-free 'Supreme' which had increased to 51% and 67%, and 'Supreme' wild-type which increased to 78% and then 91%, in November 2002 and 2003, respectively.

The mean number of pasture mealybug/core over all cultivars was significantly greater ($P < 0.001$) in nil treatments than in AR1 and wild-type with no difference between endophyte strains (Table 2). Both strains substantially reduced mealybug numbers in all cultivars with all differences being significant except those between wild-type and nil in the 'Marsden' and 'Supreme' cultivars (Table 2). Cultivar did not affect mealybug numbers, but a significant interaction between endophyte and cultivar occurred such that numbers were higher in the nil 'Nui' and 'Samson' treatments, than in nil treatments of other cultivars ($P < 0.05$).

Root aphid numbers ranged from 70/core under 'Nui' wild-type to 215/core under 'Pacific' AR1. Neither cultivar nor endophyte significantly affected numbers but there were significant differences between individual endophyte/cultivar associations ($P < 0.05$) (Table 2).

There were significant effects of cultivar and endophyte ($P < 0.001$) and of cultivar \times endophyte interactions ($P < 0.01$) on pasture productivity taken at the time of mealybug sampling in May 2001 (Table 3). Mean green yield (\pm SE) was lower in the nil cultivars (241 ± 15 kg/ha) than in the AR1 cultivars (442 ± 33.2 kg/ha) ($P < 0.001$) which in turn was lower than green yield in wild-type treatments (569 ± 30.2 kg/ha) ($P < 0.05$). Yields of 'Impact' with AR1 and wild-type and 'Samson' with wild-type were higher than yields of their counterparts in other cultivars ($P < 0.05$) (Table 3).

The growth rate in the summer/autumn period of 2001, determined from total DM yields, was lower than in the equivalent period in 2000, and this difference was significantly affected by cultivar ($P < 0.05$) and endophyte ($P < 0.001$) but not by a cultivar \times endophyte interaction. The difference in growth rate (\pm SE) over all cultivars in the nil treatments was 15.6 ± 1.34 kg/ha per day, compared with 9.9 ± 1.33 kg/ha per day in AR1 and 7.6 ± 1.30 kg/ha per day in wild-type (LSD = 3.8, $P < 0.05$). The inclusion of the proportion of endophytic plants in each treatment as a covariate explained a small component of the difference in growth rate between cultivars ($P = 0.082$), but not the difference between endophyte treatments ($P = 0.319$). Conversely, the number of mealybug/core as a covariate accounted

Table 3 Green yield determined in May 2001 and change in growth rate during the summer/autumn period between 2000 and 2001 of six different ryegrass cultivars without endophyte (nil) or infected with AR1 or wild-type endophytes.

Cultivar	Green yield (kg/ha)			Change in growth rate (kg/ha per day)		
	Nil	Wild-type	AR1	Nil	Wild-type	AR1
'Pacific'	262	561	459	11.8	8.1	7.3
'Impact'	238	727	633	17.6	11.0	9.3
'Nui'	192	581	415	15.9	2.7	7.0
'Samson'	272	719	534	11.4	4.7	8.6
'Marsden'	197	402	255	19.3	7.7	16.8
'Supreme'	284	427	359	17.7	11.1	10.3
5% LSD (d.f.)	Cultivar 82 (15) Endophyte 50 (36) Cultivar × Endophyte 125 (48)			Cultivar 4.7 (15) Endophyte 3.8 (36) Cultivar × Endophyte 50.8 (48)		

Table 4 Ryegrass cover (%) for three cultivars infected with either AR1 or wild-type endophyte or endophyte-free (Trial B; determined by point analysis May 2003).

Cultivar	Endophyte treatment		
	Nil	Wild-type	AR1
'Impact'	26	57	58
'Nui'	28	54	49
'Supreme'	31	46	47
5% LSD (d.f.)	Endophyte 3.7 (24) Cultivar 3.7 (24) Endophyte × Cultivar 6.4 (24)		

for 55% of the variation in the effect of endophyte on the change in growth rate ($P = 0.013$, Covariance Efficiency Factor = 0.45), but none of the variation in the effect of cultivar. In the nil endophyte plots alone, however, there was a significant effect of mealybug numbers on the change in growth rate between different cultivars ($P = 0.04$). Root aphid numbers were not associated with any change in yield.

Two years after the outbreak of mealybug, the mean percentage groundcover of ryegrass in plots of the three cultivars measured was 28% in the nil treatment, significantly less ($P < 0.001$) than for AR1 (51%) and wild-type (52%). Ryegrass groundcover in the nil treatments was similar for each of the cultivars, but was higher ($P < 0.01$) in 'Impact' AR1 and wild-type than for the same treatments in 'Supreme' (Table 4). 'Impact' AR1 also had more ryegrass than 'Nui' AR1 ($P < 0.05$).

DISCUSSION

This study has shown that infection of perennial ryegrass with *N. lolii* reduces infestations of pasture mealybug in the field. The reduction in numbers of this insect occurred regardless of endophyte strain, and in several different cultivars. For the two novel strains of endophyte, AR1 and AR37, resistance to pasture mealybug was as strong as that shown by the wild-type endophyte. Similarly, a range of different strains of *Neotyphodium* spp. in tall fescue (*Festuca arundinacea* Schreb.) have also been shown to substantially reduce mealybug numbers (Pennell & Ball 1999).

Infestations of pasture mealybug originate from passive movement of crawlers, and hence their ability to select suitable hosts is limited, although first and second instars may feed at more than one site before becoming established. Pearson (1988) attributed the endophyte effect on pasture mealybug that he demonstrated in pot trials to a failure of this crawler stage to establish. That effect is almost certainly mediated by alkaloids produced by the fungus which may influence crawler selection through deterrence (antixenosis) and/or by direct toxicity (antibiosis). The different alkaloid profiles of the strains evaluated in the trials reported here mean we cannot conclusively identify the chemical basis of the resistance. Peramine, for instance, is a powerful feeding deterrent to Argentine stem weevil (Rowan et al. 1990). This alkaloid is present in ryegrass infected with either AR1 or wild-type and, therefore, may have a role in reducing mealybug infestations.

There is also the possibility that an unidentified factor present in AR1 which deters black beetle (Popay & Baltus 2001) is responsible for the effect of this endophyte on pasture mealybug. AR37 produces none of the alkaloids known to be produced by the wild-type and AR1, but produces a complex of janthitrems (Tapper & Lane 2004), although we cannot be sure that these are responsible for the anti-insect activity observed.

Neither AR1 nor wild-type endophytes reduced populations of root aphid in the field. In Trial A, results indicated a slight increase in susceptibility of AR1-infected 'Nui' ryegrass to root aphid compared with endophyte-free ryegrass, but this did not occur in Trial B. The data on root aphid populations reported in this paper need to be interpreted with some caution. Hand sorting of samples in Trial A would have resulted in many immature root aphid not being seen, so the numbers presented are likely to be a considerable underestimation of the actual population. In Trial B, wet sieving captured all life stages of the aphid, but samples were taken only to a depth of 100 mm in this trial (40 mm in Trial A) and root aphid can be found at greater depths than this. Nevertheless, the data in Trial A suggested that one endophyte, AR37, may reduce root aphid numbers, and this has also been demonstrated in other studies (Popay et al. 2004). Root aphid is known to be adversely affected by the presence of *N. uncinatum* in meadow fescue (*F. pratensis*) (Schmidt & Guy 1997) and by *N. coenophialum* in tall fescue (A. J. Popay & C. G. L. Pennell unpubl.), due probably to the presence of loline alkaloids in these associations, which are known to affect other aphid species (Siegel et al. 1990). There was some evidence that root aphid contributed to reduced pasture productivity in Trial A in May 1998 but not in Trial B. Cottier (1953) considered this aphid to be of no economic importance but, given the high populations of this insect in the field, further studies may be warranted. In Jordan, this root aphid has been recorded as seriously damaging early-sown wheat (Mustafa & Akkawi 1987).

Densities of mealybug in endophyte-free ryegrass in Trial A were 844/m², while in unreplicated strips of 'Nui' in a paddock close to Trial A, densities were estimated to be 11 710/m². Wilting and discolouration of the grass, and death of tillers, the visible symptoms of damage which result from such high densities, appear in late autumn and resemble drought stress. Such symptoms were apparent in both trials, and the concurrent reductions in pasture productivity were related to the presence of the

pasture mealybug. Even so, both endophyte strain and cultivar were also significant contributors to yield differences, particularly in Trial B. No other insect pests were present which could account for yield differences. Argentine stem weevil damage does not occur in late autumn, and the damage symptoms were distinct from those caused by root-feeding grass grub larvae (*Costelytra zealandica* (White)) or the foliar-feeding porina caterpillars (*Wiseana* spp.). In Trial A, there were no apparent long-term effects of insect damage on pasture productivity and by spring endophyte-free plots had recovered (Fig. 1). This was not the case in Trial B where considerable plant mortality occurred in nil treatments resulting in 25% less ryegrass in these plots 2 years after the outbreak. In addition, the increase in infection frequency in nil 'Supreme' from 26% in November 2000 to 51% in 2003 is likely to have been caused by mealybug-induced mortality of endophyte-free plants.

Other studies have also demonstrated that infestations by mealybug can damage grasses. Pearson (1988) found that in two out of six harvests, productivity of potted ryegrasses was reduced by high infestations of pasture mealybug. This author also recorded significant increases in DM production of endophyte-infected over endophyte-free ryegrass in four of six harvests, and attributed this to differential feeding by pasture mealybug. Studies in the United States have shown that other mealybug species, including the Rhodesgrass mealybug (*Antonina graminis* (Maskell)), and the buffalograss mealybugs (*Tridiscus sporoboli* (Cockerell) and *Trionymus* sp.) are also capable of seriously damaging pasture and turf grasses (Tashiro 1987; Baxendale et al. 1994; Baxendale & Shetlar 1995; Johnson-Cicalese et al. 1998).

Damage to grasses inflicted by mealybug in most instances becomes more apparent during long dry spells (Tashiro 1987; Pearson 1988; Baxendale & Shetlar 1995) and this was the case in the Lincoln trials. Long-term mean monthly rainfall between January and May at Lincoln is 49 mm. During the outbreak of mealybug in Trial A in 1998 and Trial B in 2001 the means for the same period were 26 and 20 mm, respectively, while for this period in both years preceding these outbreaks, rainfall was 56 mm. The exacerbation of damage during drought probably results from sap-sucking by the mealybug which disrupts the vascular tissues and impedes translocation of water and nutrients. We observed that damage was noticeably reduced on a strip of endophyte-free pasture at Lincoln which

was unintentionally irrigated and, in addition, that dry soils appear able to support greater numbers of pasture mealybug than moist soils. Irrigation is known to help prevent damage to turf or pastures by another mealybug species, *A. graminis* (Tashiro 1987). Pasture mealybug damage may also be prevented by break feeding of stock at critical times in early summer to prevent crawler establishment (C. G. L. Pennell unpubl. data).

This study has presented strong evidence implicating pasture mealybug as the agent responsible for damaging endophyte-free ryegrass trials at Lincoln. There have been few reports of this pest outside the Lincoln area, but this may be because the symptoms of damage resemble, and coincide with, those of drought. Two new strains of fungal endophyte, AR1 and AR37, provide their host ryegrass with strong protection against this pest. The role that root aphid may have in reducing pasture productivity, however, needs further investigation.

ACKNOWLEDGMENTS

We thank H. S. Easton and N. Cox, AgResearch Palmerston North and Ruakura for help with statistical analyses; D. Pearson, National Plant Pest Reference Laboratory, M. A. F., Lincoln; and M. Dyksma for his technical assistance.

REFERENCES

- Baxendale FP, Shetlar DJ 1995. Mealybugs. In: Brandenburg RL, Villani MG ed. Handbook of turf insect pests. Lanham, MD. Entomological Society of America. Pp. 76–77.
- Baxendale FP, Johnson-Cicalese JM, Riordan TP 1994. *Tridiscus sporoboli* and *Trionymus* sp. (Homoptera: Pseudococcidae): potential new mealybug pests of buffalograss turf. Journal of the Kansas Entomological Society 67: 169–172.
- Cottier W 1953. Aphids of New Zealand. Wellington, New Zealand. New Zealand Department of Scientific and Industrial Research Bulletin 106. 382 p.
- Cox JM 1987. Pseudococcidae (Insecta: Hemiptera). Fauna of New Zealand No. 11.
- de Boer JA 1968. Taxonomy of the New Zealand Pseudococcidae, Part 2. (Homoptera: Coccoidea). New Zealand Journal of Science 11: 328–336.
- Earle DF, McGowan AA 1979. Evaluation and calibration of an automated rising plate meter for estimating dry matter yield of pasture. Australian Journal of Experimental Agriculture and Animal Husbandry 19: 337–343.
- Fletcher LR 1999. “Non-toxic” endophytes in ryegrass and their effect on livestock health and production. In: Woodfield DR, Matthew C ed. Ryegrass endophyte: an essential New Zealand symbiosis. Grassland Research and Practice Series No. 7: 133–139.
- Fletcher LR, Sutherland BL, Fletcher CG 1999. The impact of endophyte on the health and productivity of sheep grazing ryegrass-based pastures. In: Woodfield DR, Matthew C ed. Ryegrass endophyte: an essential New Zealand symbiosis. Grassland Research and Practice Series No. 7: 11–17.
- Gourly CJP, McGowan AA 1991. Assessing differences in pasture mass with an automated rising plate meter and a direct harvesting technique. Australian Journal of Experimental Agriculture 31: 337–339.
- Hahn H, Huth W, Schoberlein W, Diepenbrock W 2003. Detection of the endophytic fungi in *Festuca* spp. by means of tissue print immunoassay. Plant Breeding 122: 217–222.
- Mustafa TM, Akkawi M 1987. The occurrence, economic importance and control of wheat root aphid (*Aploneura lentisci* Passerini, Homoptera, Aphididae) on wheat in Jordan. Disarat 2: 83–88.
- Johnson-Cicalese J, Baxendale F, Riordan T, Heng-Moss T 1998. Identification of mealybug- (Homoptera: Pseudococcidae) resistant turf-type buffalograss germplasm. Journal of Economic Entomology 91: 340–346.
- Pearson WD 1988. The pasture mealy bug, *Balanococcus poae* (Maskell), in Canterbury: a preliminary report. In: Stahle PP ed. Proceedings of the 5th Australasian Conference on Grassland Invertebrate Ecology. Pp. 297–303.
- Pennell C, Ball OJ-P 1999. The effects of *Neotyphodium* endophytes in tall fescue on pasture mealy bug (*Balanococcus poae*). Proceedings of the 52nd New Zealand Plant Protection Conference. Pp. 259–263.
- Pennell CGL, Popay AJ, Baxendale FP 2004. The pasture mealybug *Balanococcus poae* (Maskell) in New Zealand pastures. In: Winder LM, Goldson SL ed. Proceedings of the 8th Australasian Conference on Grassland Invertebrate Ecology. Pp. 80–86.

- Popay AJ, Baltus JG 2001. Black beetle damage to perennial ryegrass infected with AR1 endophyte. Proceedings of the New Zealand Grassland Association 63: 267–271.
- Popay AJ, Wyatt RT 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 AJ, Hume DE, Baltus JG, Latch GCM, Tapper BA, Lyons TB, Cooper BM, Pennell CG, Eerens JPJ, Marshall SL 1999. Field performance of perennial ryegrass (*Lolium perenne*) infected with toxin-free fungal endophytes (*Neotyphodium* spp.). In: Woodfield DR, Matthew C ed. Ryegrass endophyte: an essential New Zealand symbiosis. Grassland Research and Practice Series No. 7: 113–122.
- Popay AJ, Silvester WB, Gerard PJ 2004. New endophyte isolate suppresses root aphid, *Aploneura lentisci*, in perennial ryegrass. In: Kallenbach R, Rosenkrans CJ, Lock TR ed. 5th International Symposium on *Neotyphodium*/Grass Interactions. Fayetteville, Arkansas. P. 317.
- Rowan DD, Dymock JJ, Brimble MA 1990. Effect of fungal metabolite peramine and analogs on feeding development of Argentine stem weevil (*Listronotus bonariensis*). Journal of Chemical Ecology 16(5): 1683–1695.
- Schmidt D, Guy PL 1997. Effects of the presence of the endophyte *Acremonium uncinatum* and of an insecticide treatment on seed production of meadow fescue. Revue Suisse d'Agriculture 29: 97–99.
- Siegel MR, Latch GCM, Bush LP, Fannin FF, Rowan DD, Tapper BA, Bacon CW, Johnson MC 1990. Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. Journal of Chemical Ecology 16(12): 3301–3315.
- Tapper BA, Lane GA 2004. Janthitrems found in a *Neotyphodium* endophyte of perennial ryegrass. In: Kallenbach R, Rosenkrans CJ, Lock TR ed. 5th International Symposium on *Neotyphodium*/Grass Interactions. Fayetteville, Arkansas. P. 301.
- Tashiro H 1987. Turfgrass insects of the United States and Canada, Cornell University Press. New York, Ithaca.