THE EFFECTS OF *NEOTYPHODIUM* ENDOPHYTES IN TALL FESCUE ON PASTURE MEALY BUG (*BALANOCOCCUS POAE*)

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ABSTRACT

The effect of different *Neotyphodium* endophyte strains in novel and natural tall fescue (*Festuca arundinacea*) associations on pasture mealy bug (*Balanococcus poae*) was investigated under grazing. All of the endophyte-infected tall fescue treatments had significantly lower numbers of mealy bugs compared to an endophyte-free control. Resistance to pasture mealy bug was maintained in novel associations free of the mammalian toxin ergovaline. Point analysis revealed that endophyte-free plots contained less tall fescue herbage than endophyte-infected treatments. This paper raises the possibility that the pasture mealy bug was responsible.

Keywords: Endophyte, *Neotyphodium*, pasture mealy bug, *Balanococcus poae*, tall fescue.

INTRODUCTION

In most areas of New Zealand, pastoral farming is heavily dependent upon the presence of perennial ryegrass (*Lolium perenne*). Tall fescue (*Festuca arundinacea*) is used extensively in South Eastern USA and has recently been revived as alternative forage for dry regions of New Zealand. Both species are often infected with endophytic fungi from the genus *Neotyphodium* (formerly *Acremonium*), perennial ryegrass with *N. lolii* and tall fescue with *N. coenophialum*. The presence of these endophytes is important because infected plants are resistant to attack from many insect pests (Popay and Rowan 1994). However, livestock feeding on pastures containing predominantly wild type endophyte infected plants may suffer health problems, including ryegrass staggers in perennial ryegrass pastures (Fletcher and Harvey 1981) and fescue toxicosis in tall fescue pastures (Bacon *et al.* 1977).

Insect resistance and animal health effects are thought to be due to the presence of alkaloids in infected plants. Four major alkaloids or groups have been identified. Peramine is the major Argentine stem weevil (*Listronotus bonariensis*) feeding deterrent in ryegrass (Rowan and Gaynor 1986), and lolitrem B is thought to be responsible for causing ryegrass staggers (Gallagher *et al.* 1981). Ergovaline, which is found in most ryegrass and tall fescue endophyte associations, is the likely cause of heat stress and fescue toxicosis (Fletcher and Easton 1997), while the lolines have been identified as important insect resistance factors (Dahlman *et al.* 1991).

Compared to other New Zealand pasture pests, the pasture mealy bug (*Balanococcus poae*) has received little attention from researchers. The pasture mealy bug has been reported on many species of native grass, as well as perennial ryegrass, cocksfoot (*Dactylis glomerata*) and Yorkshire fog (*Holcus lanatus*) (Cox 1987). In the South Island, we have also found pasture mealy bug on tall fescue, timothy (*Phleum pratense*) and chewings fescue (*Festuca rubra*, sub sp. *commutata*). There have been no reports of serious infestations in the North Island. Pasture mealy bug has not generally been considered a serious problem in pasture. However, in 1998, deterioration of endophyte-free perennial ryegrass plots relative to endophyte-infected plots in evaluation trials at Lincoln, was observed and linked to the presence of the pasture mealy bug (C. Pennell unpubl. data). A similar situation in a trial examining the

performance of different novel and natural tall fescue-endophyte associations was observed in March 1999. Assessments of pasture mealy bug levels and ground cover were therefore undertaken.

METHODS

The effect of *Neotyphodium* infection in tall fescue on pasture mealy bug infestations was investigated using four different endophytes, AR501, AR502, AR542 and wild type *N. coenophialum*. AR501, AR502 and AR542 were isolated from tall fescue plants grown from seed collected in Spain and Morocco, and inoculated into tall fescue cultivars developed at AgResearch using the method of Latch and Christensen (1985). AR542 and the wild type endophyte are true representatives of the species *N. coenophialum*. Although derived from tall fescue, AR501 and AR502 are classified as *Festuca arundinacea* Taxonomic Grouping 3 (FaTG-3) endophytes rather than *N. coenophialum* (Christensen *et al.* 1993). AR501, AR502 and AR542 were selected, because they do not produce ergovaline which is the alkaloid suspected of causing fescue toxicosis in livestock. The alkaloid profiles of each association are presented in Table 1.

TABLE 1: Alkaloid profiles of tall fescue-*Neotyphodium* associations used in this study.

<i>Neotyphodium</i> isolate	Taxon ¹	Tall Fescue	Peramine	Ergovaline	Lolines ²
AR501	FaTG-3	Advance Sel.	+	-	+
AR502	FaTG-3	Advance Sel.	+	-	+
AR542	<i>N. coenophialum</i>	Experimental	+	-	+
Wild type	<i>N. coenophialum</i>	Kentucky 31	+	+	+

¹Christensen *et al.* (1993). FaTG-3 = *Festuca arundinacea* Taxonomic Grouping 3.

²AR542 associations do not contain the known insect active loline alkaloids (*N*-acetyl loline and *N*-formyl loline) but instead contain *N*-acetyl norloline.

In March 1997, four replicate plots (5 x 3m) at two Lincoln sites (a high fertility deep Wakanui silt loam at site A, and a low fertility shallow Lismore silt loam at site B) were sown at 20 kg/ha. At sites A and B, seed derived from plants of 'Grasslands Advance' (Advance sel.) inoculated with endophyte strains AR501 and AR502, and 'Kentucky 31' infected with the wild type endophyte, were sown. An endophyte-free 'Advance' selection was sown as a control at both sites. At site B, two plots (1 x 5m) of an experimental tall fescue line infected with AR542 were also sown. Endophyte infection levels in each plot were monitored using tissue print immunoblot (Gwinn *et al.* 1991).

Assessment of pasture mealy bug abundance

In March 1999, 16 randomly positioned turf cuts per plot, exposing the crown and root area of individual plants, were made at both sites, as destructive sampling was not possible. Each turf cut was assessed for the presence or absence of pasture mealy bug. The presence of mealy bug was verified by observation of individual insects, or by the occurrence of the associated flocculent white wax. Data were analysed using a Chi-squared test.

Assessment of ground cover and dry matter yield.

A string line consisting of 100 points running diagonally from one corner of each plot to the other was used to assess ground cover by point analysis. The presence of bare ground, live tall fescue herbage, or dead herbage at each point along the string line was noted. Both diagonals in each plot were assessed. A Chi-squared analysis was performed on the data.

Dry matter yields were obtained by taking sub samples of herbage from each plot. Herbage was oven dried and weighed

RESULTS

Percentage endophyte infection

Percent endophyte infection in plots containing AR501, AR502, AR542 and wild type endophytes, was at least 62% (Table2). The percentage of tall fescue plants containing endophyte from control plots was 0%.

TABLE 2: Percentage endophyte infection and mealy bug infestation.

Tall Fescue	Endophyte	% Endophyte infection 1998		% Mealy bug infestation	
		Site A	Site B	Site A	Site B
Advance selection ¹	Nil control	0	0	91	75
Advance selection	AR501	78	71	11	11
Advance selection	AR502	66	62	2	2
Experimental	AR542	-	100	-	9
Kentucky 31	Wild type	80	79	9	5

¹Advance sel. nil control different from all others Chi-square $P < 0.001$ $P < 0.001$

Pasture mealy bug infestation

At both sites, plants from endophyte-free tall fescue plots were significantly ($P < 0.001$) more infested with mealy bug than plants in all endophyte-infected treatments (Table 2). There were no significant differences in the percentage of plants infested with mealy bug between any of the endophyte-infected treatments.

Assessment of ground cover and dry matter yield

There was significantly ($P < 0.001$) more bare ground and less tall fescue herbage in endophyte-free plots compared with all endophyte-infected plots (Table 3). The proportion of bare ground and tall fescue herbage did not differ significantly between endophyte-infected treatments at either site. The 1998/99 summer dry matter yield data gave a 20% increase in production at site A and a 42% increase in site B in favour of the endophyte-infected treatments.

TABLE 3: Estimate of ground cover using point analysis.

Tall Fescue	Endophyte	Site A			Site B		
		Green	Bare	Dead	Green	Bare	Dead
Advance sel.	Nil control	50	46	5	24	70	6
Advance sel.	AR501	62	35	3	42	53	6
Advance sel.	AR502	59	40	2	40	56	4
Experimental	AR542	-	-	-	52	45	4
Kentucky 31	Wild type	59	40	1	43	52	6

Advance sel. nil control different from all others Chi-square

$P < 0.001$

$P < 0.001$

DISCUSSION

This study has shown that *Neotyphodium* endophyte infection protects tall fescue against attack from the pasture mealy bug. Resistance against this pest appears to be a common property of *Neotyphodium* endophytes derived from tall fescue, as the abundance of mealy bug was significantly lower in all endophyte-infected plots compared with endophyte-free plots. However, it should be noted that three different tall fescue cultivars were used in the study, and only one of these was endophyte-free. Thus, there is a possibility that plant genotype differences could account for some of the differences in mealy bug abundance. However, evidence for a potent endophyte

effect is strong, as strains AR501 and AR502 were present in the 'Advance' tall fescue selection for which there was an endophyte-free control.

Pot trials have shown that endophyte-infected perennial ryegrass suffered less pasture mealy bug damage than endophyte-free ryegrass (Pearson 1988). The same effect against pasture mealy bug was observed in the field with perennial ryegrass infected with different strains of *N. lolii* (C. Pennell, unpubl. data).

The artificial derivation of three of the associations (AR501, AR502 and AR542) in the present study did not appear to weaken the adverse effect of the endophytes against pasture mealy bug. This is a significant result, as these three associations expressed atypical alkaloid profiles compared to the wild type association. Specifically, associations containing AR501, AR502 and AR542 did not contain ergovaline, the alkaloid implicated as the cause of fescue toxicosis and heat stress problems in livestock (Fletcher and Easton 1997). Thus, it appears possible to develop novel tall fescue-endophyte associations that do not cause health problems in grazing animals, but which are resistant to pasture mealy bug.

Point analysis and dry matter yield estimates showed that there was less tall fescue herbage and more bare ground in endophyte-free plots than in all endophyte-infected treatments. Pulling by stock after grazing was noted, probably contributing to reduced plant populations in endophyte-free plots. It is possible to speculate about the causes of these differences, but there is evidence to suggest that pasture mealy bug was the major factor. Firstly, tillers from the outer crowns where mealy bug were predominantly found, were generally affected to a greater degree than the innermost tillers. This was confirmed on an area where destructive sampling was possible and plants dissected. Secondly, there was no evidence for the activity of any other insect pest. For example, damage was not typical of grass grub (*Costelytra zealandica*) feeding. Also, there was no indication that damage to tall fescue plants was inflicted by larvae of the Argentine stem weevil. Even when free of endophyte, tall fescue is not susceptible to Argentine stem weevil larval attack (Goldson 1982).

It is not possible to conclusively attribute the reduction in ground cover in the present trial to mealy bug alone. West and Gwinn (1993) showed that endophyte infection improved the drought tolerance and plant density of tall fescue. However, these results, together with previous observations on ryegrass plots, indicate that the pasture mealy bug could be an important factor in pasture deterioration in certain areas of New Zealand. Pastures under stress from low fertility, drought, insect attack or undue grazing management may be more susceptible. Additional research to confirm this is therefore required.

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