

The effect of non-toxic endophytes in tall fescue on two major insect pests

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Abstract

In a choice trial in a glasshouse, infection of tall fescue with the endophyte AR542 significantly reduced feeding by adult Argentine stem weevil (ASW) in the cultivar Jesup but not in Advance in comparison with the same cultivars without endophyte. Paddocks of Quantum infected with AR542 that were sampled on two Northland farms consistently had fewer tillers damaged by Argentine stem weevil than equivalent paddocks of endophyte-free (Nil) tall fescue. In comparison to Nil plants, AR542 in Jesup and Advance had significantly fewer plants and tillers damaged by adult black beetle in a glasshouse choice trial. Fewer tillers were also damaged by adult black beetle in Advance tall fescue infected with AR501 and AR502 than in Nil Advance in a small plot field trial at Kerikeri. In a similar trial at Ruakura, the occurrence of adult black beetle and signs of larval damage were high in endophyte-free plots and considerably reduced in endophyte-infected plots. It is concluded that the non-toxic endophytes will lessen damage by black beetle and ASW in a range of tall fescue cultivars and that this is likely to contribute to increases in productivity and persistence of this forage grass.

Keywords: Argentine stem weevil, black beetle, AR542 endophyte, *Neotyphodium*, tall fescue

Introduction

Tall fescue (*Festuca arundinacea*) is a valuable forage grass in New Zealand, particularly in areas where high summer temperatures and moisture stress limit the growth of perennial ryegrass (*Lolium perenne*). Tall fescue pastures are capable of higher summer and annual growth than perennial ryegrass (McCallum *et al.* 1992; Easton *et al.* 1994) and have a higher clover content than ryegrass pastures infected with Wild-type endophyte (Exton *et al.* 1996). On a dairy farm in the Waikato, a 16% increase in milk production was achieved from cows grazing tall fescue compared with a similar herd on ryegrass-based pastures (Milne *et al.* 1997). In contrast to this, however, McCallum *et al.* (1992) concluded that tall fescue was unsuitable for use under intensive dairying in Taranaki except in areas prone to dry summers and frequent damage by grass grub (*Costelytra zealandica*). Tall fescue is slower to establish than ryegrass and requires careful management to realise its full potential.

Although more tolerant of grass grub damage (Kainet *et al.* 1979; East *et al.* 1980) and less susceptible to Argentine stem weevil (*Listronotus bonariensis*) damage compared with ryegrass (Prestidge *et al.* 1986), tall fescue is not immune from damage by these pests. In addition, black beetle (*Heteronychus arator*), a major pest of grasses in northern North Island areas, can severely limit growth and longevity of tall fescue pastures.

In the south-eastern United States, where tall fescue is the most widely grown cool-season forage grass, infection by the fungal endophyte *Neotyphodium coenophialum* is considered essential for maintaining pasture productivity and persistence despite the fescue toxicosis it causes in livestock grazing infected pastures. A similar toxicosis can occur in NZ when animals graze on wild tall fescue which is infected with a toxic endophyte. In 2000, a non-toxic *Neotyphodium* endophyte, AR542 (Max Q™) was released commercially into the United States market after extensive testing showed that agronomic performance of tall fescue infected with this endophyte was similar to that of tall fescue infected with the Wild-type endophyte without the adverse effects on cattle and sheep caused by the latter (Bouton *et al.* 2002; Parish *et al.* 2003). This endophyte AR542 (as Max P™) is now available to farmers throughout Australasia in several tall fescue cultivars. The effect of AR542 and other non-toxic endophytes on two major insect pests, ASW and black beetle has been evaluated in NZ. This paper reports the results of that research.

Methods

Argentine stem weevil

The effect on adult ASW feeding of the endophyte AR542 in young plants of two cultivars of tall fescue, Advance and Jesup, was assessed in a choice trial with endophyte-free plants, carried out in a glasshouse. An additional three experimental endophytes in Jesup were also evaluated alongside the AR542 but the results for these treatments will not be reported here. For each replicate, germinated seed of each treatment was planted in rows of eight plants in a seed tray containing commercial potting mix on 29 March 2004. Five replicate seed trays were prepared for each treatment with the placement of treatments randomised within each tray. Spare plants were planted into large

polystyrene seed boxes containing the same potting mix. All plants were kept in a glasshouse under natural light and with regular watering.

At the end of April, seedlings that had not grown were replaced with spares and all plants were tested for endophyte presence using tissue immunoprint assay (Hahn *et al.* 2003). On 4 May, 53 adult ASW were released onto each tray and confined using fine nylon net enclosures. The weevils had been collected from the field near Mosgiel in the South Island in October 2003 and January 2004 and maintained in the laboratory.

Adult ASW feeding was assessed on each plant on 19 May, 2 weeks after they were released. Leaf blades on each tiller (2–4 tillers/plant) were visually inspected and each plant assigned a feeding damage score on a scale of 0–5 where 0 = no feeding and 5 = extensive feeding scars present on all leaf blades. An analysis of variance was carried out on untransformed data using a general linear model. Data for plants of each treatment which did not have the appropriate endophyte status were excluded from the analysis.

Field evaluations of ASW larval damage were made on adjacent unreplicated paddocks of tall fescue cv. Quantum without endophyte or infected with AR542 on two Northland farms. One site near Dargaville, comprising 7 ha each of Nil and endophyte-infected fescue, was sown in spring 2001. The other, at Ohaeawai, west of Kerikeri, was sown at the end of May 2001 and consisted of 5.5 ha of each. In late November 2001 and again in February 2002, 100 tillers were randomly taken from each paddock. These were dissected and ASW larval damage recorded. At the Dargaville site, samples were taken as clumps of tillers which allowed dead tillers to be removed and dissected separately from the live tillers.

Black beetle

The choice seed tray trial for ASW described above was also used to test the response of adult black beetle to AR542 in Jesup and Advance. After completion of the ASW trial, plants that were free from endophyte in the endophyte-infected treatments were removed from each row. Dead sheath material and tillers were removed from remaining plants which were then trimmed to about 7 cm. A liquid fertiliser was applied to each row of seedlings and acetate sheets put in place around the trays to prevent beetles escaping. On May 25, 10 adult beetles, field-collected from Northland over the previous weeks, were released onto each of the five replicate trays. Two weeks later on June 10, the number of damaged and undamaged tillers on each plant was recorded. Damaged tillers were recorded as being either slightly damaged where there were small feeding scars at the base of the tiller or severely damaged where the

base of tillers was completely shredded and the tiller was dead or dying.

Field trials with non-toxic endophytes were also sampled for black beetle adult and larval damage. In autumn 1997, small plot field trials of tall fescue were sown at the AgResearch research stations at Ruakura and Kerikeri. Among the treatments in these trials were Advance without endophyte and Advance infected with the non-toxic endophytes AR501 and AR502. There were four replicate plots of each treatment arranged in a randomised block design. Both sites were sampled in autumn 1999. At Ruakura, four spade squares were taken from each of three replicate plots of each treatment and searched for presence of adult black beetle and signs of larval damage ie. presence of tunnelling beneath plants and frass that is characteristic of black beetle larvae. At Kerikeri, a minimum of 30 live tillers were cut at the base from each of the four replicate plots for each treatment and examined for black beetle adult damage. All dead tillers sampled were also examined.

In addition, 70 live tillers were harvested in March 2004 from each of the adjacent AR542 and endophyte-free tall fescue paddocks on the Ohaeawai farm. These, plus all dead tillers sampled at the same time, were examined for symptoms of adult black beetle feeding.

Untransformed data on the percentage of plants and tillers damaged in the glasshouse trial were analysed as binomial data using regression analysis with a logit link function which took into account the number of plants and tillers in each treatment. The data from the replicated small plot field trials were analysed using ANOVA.

Results

Argentine stem weevil

In the glasshouse trial, endophyte infection levels in Advance with AR542 were 67.5% (27 of 40 plants were infected) and 100% in Jesup. No endophyte infection was found in Nil treatments of either cultivar. Plants in the Advance AR542 treatment that were endophyte-free were not removed prior to assessment of ASW feeding, but the data for these plants were excluded from the analysis. Adult weevil feeding damage (based only on plants with the correct endophyte status) was significantly less on endophyte-infected tall fescue plants than on endophyte-free, for both cultivars combined ($P<0.01$) and for Jesup alone ($P<0.001$) (Table 1). Feeding score on Advance AR542 was not significantly less than that on Nil Advance ($P>0.05$). There was no significant effect of cultivar itself on weevil feeding.

At the Dargaville site, ASW larval damage was recorded in 13% of endophyte-free tillers sampled in November and in 8% of tillers sampled in February. In the February sample, there were also 11 dead tillers and

Table 1 Argentine stem weevil adult damage (mean feeding score per plant) on two cultivars of tall fescue without endophyte (Nil) or infected with the endophyte AR542.

Cultivar	Endophyte		SED
	AR542	Nil	
Advance	0.938	1.35	Endophyte 0.185
Jesup	0.425	1.35	Cultivar 0.185
Mean	0.682	1.35	Endophyte*Cultivar 0.26

Table 2 Percentage of plants and tillers damaged by adult black beetle given a choice between two cultivars of tall fescue either without endophyte (Nil) or infected with AR542. Total numbers of plants and tillers assessed for damage are given in parenthesis.

Cultivar	% Plants damaged (N)		% Tillers damaged (N)	
	AR542	Nil	AR542	Nil
Advance	0 (27)	58 (38)	0 (106)	42 (133)
Jesup	8 (37)	27 (40)	3 (127)	19 (187)
Mean	4 (64)	41 (78)	2 (233)	39 (320)
Average SED		6.7		3.3

9 of these (82%) had been killed by stem weevil larvae. Larval damage to tillers from AR542-infected fescue paddocks was 1% on both sampling occasions. In the February sample only 3 dead tillers were collected and none of these showed signs of larval damage. Levels of larval damage on the Ohaeawai farm were low with 5% of Nil tillers sampled in November damaged and only 1% in February. No damage was found in the samples taken from the AR542-infected paddock.

Black beetle

In the glasshouse choice trial, there were far fewer plants and tillers damaged by adult black beetle in the AR542 treatment than in Nil for both cultivars ($P<0.001$) (Table 2). There was a significant cultivar*endophyte interaction such that Nil Advance tall fescue suffered significantly more damage (both plants and tillers) than Nil Jesup ($P<0.001$), whereas there was no difference in damage between these two cultivars when they were infected with AR542 ($P>0.05$). Of the Nil tillers that were damaged in Jesup ($N=35$) and Advance ($N=55$), 86% and 84%, respectively were severely damaged, whereas only one of the four tillers damaged in Jesup AR542 was classed as severely damaged.

In the Kerikeri small plot trial, adult black beetle damage was recorded in 23% of Nil tillers and 3% and 7% of tillers in plots infected with AR501 and AR502 respectively ($P<0.01$). In the equivalent trial at Ruakura, the percentage of samples taken which contained adult black beetle was 83% in Nil, 0% in AR501 and 17% in AR502-infected tall fescue ($SED = 16.67$, $P<0.05$). Similarly, signs of larval damage were present in 75% of samples taken in Nil plots but only in 8% of samples

from each of the two endophyte-infected treatments ($SED = 6.8$, $P<0.001$).

In the unreplicated paddocks of tall fescue at Ohaeawai, 23% of 70 tillers taken in March 2004 from the endophyte-free fescue were damaged by adult black beetle compared with only 1% of tillers infected with AR542. No dead tillers were among the samples taken from the AR542-infected paddock but six of 10 dead tillers sampled from the Nil paddock were damaged by adult black beetle and a further three by ASW larvae.

Discussion

Deterrency to adult feeding is the basis of the resistance to black beetle and ASW conferred by many endophytes. Adequate food resources are essential for sustaining adult black beetle survival through autumn and winter and allowing them to lay eggs in spring while deterrency of adult ASW also reduces egg lay and hence damage by the destructive larval stage. These trials have shown that AR542 in the cultivars Jesup and Advance reduces adult black beetle feeding both in the laboratory and in the field. Resistance to adult black beetle from infection by AR542 has also been demonstrated previously in the cultivar Kentucky 31 (Popay 2004). The resistance to adult black beetle was reflected in far fewer signs of larval damage in endophyte-infected plots in the field trial at Ruakura compared with the endophyte-free plots which were almost destroyed by black beetle larval damage during the summer and autumn of 1999.

Laboratory testing also showed that AR542 significantly reduces adult ASW feeding in the cultivar Jesup but the results were less certain for Advance. This may reflect a lower level of the active factors that deter

ASW in Advance compared with Jesup. Alternatively, weevil behaviour may have been altered in the Advance AR542 treatment by one third of the plants in these rows being endophyte-free, thus giving the weevils a less definitive choice compared with Jesup AR542 where all plants were infected. As the samples taken from the Northland farms indicate, AR542 in Quantum is also effective at reducing ASW larval damage. Whether this is due, at least in part, to metabolites produced by the endophyte acting directly on the larvae, as well as deterring the adults, has yet to be determined. Further work is also needed to demonstrate that there is robust resistance to ASW in Advance with AR542 in the field.

The non-toxic endophytes in tall fescue do not produce the ergopeptine alkaloids that are believed to be responsible for fescue toxicosis in livestock in the United States (Hill *et al.* 1994) but which also deter feeding by adult black beetle (Ball *et al.* 1997). They do, however, produce derivatives of the lolaine alkaloids which are known to have broad spectrum activity against several insects (Popay & Bonos 2004). These metabolites may be responsible for mediating the observed resistance to both black beetle and ASW in tall fescue infected with AR542, AR501 or AR502, but we cannot be sure of this until assays with lolaine derivatives are conducted on these insects. These endophytes also produce peramine which is a potent deterrent to ASW but which has no effect on black beetle. The concentrations of peramine in tall fescue infected with these endophytes, however, are considerably lower than the concentrations produced by, for example, the AR1 and Wild-type endophytes in ryegrass (D.E. Hume, unpublished data) and may be too low to be biologically active against the weevil.

Endophyte infection of tall fescue increases its productivity and persistence, an effect which, at least partially, can be attributed to a reduction in insect pests, although in the United States the endophyte is also considered to protect its host from abiotic stress such as drought. Here in NZ, both ASW and black beetle are capable of causing damage to tall fescue. In a survey of Roa tall fescue, ASW were present in all pastures with up to 39% of tillers killed by larvae (Prestidge *et al.* 1989). Higher yields and plant populations have been measured in tall fescue infected with the endophytes AR501 and AR502 compared with endophyte-free plots in the small plot trial at Kerikeri where black beetle were present (Cooper *et al.* 2002) and tiller counts taken in March 2004 on the farm at Ohaeawai found nearly twice as many tillers in the paddocks infected with AR542 as in the endophyte-free (B.M. Cooper unpublished data). In New South Wales, dramatic differences in productivity between tall fescue without endophyte or infected with AR542 were measured in both Advance

and Quantum and were attributed to severe black beetle larval damage to endophyte-free plots within a year of sowing (Wheatley *et al.* 2003). Similarly in the Ruakura small plot trial, yield in endophyte-free plots was 316 kg DM/ha compared with 1154 and 1426 kg DM/ha in the AR501 and AR502 plots respectively in May 1999 (D.E. Hume unpublished data) following major black beetle damage during the summer and autumn. In addition to their effects on black beetle and ASW, these non-toxic endophytes in tall fescue can reduce populations of pasture mealybug (*Balanococcus poae*) (Pennell & Ball 1999) and the root aphid, *Aploneura lentisci* (Popay & Jensen 2005).

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