Endophyte effects on major insect pests in Waikato dairy pasture

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Abstract

A paddock-scale field trial comparing pastures of perennial ryegrass without endophyte (Nil) or infected with the selected endophytes AR1, AR37 or the standard endophyte (SE) sown with clover was monitored for insect pests over 3 years (2006–2008). Argentine stem weevil larval damage was reduced by all endophytes in each year. Black beetle numbers remained low in AR37, SE and AR1 pastures, except in the third year when numbers showed an upward trend in AR1 pastures. Nil pastures always contained the highest black beetle numbers, significantly higher than in AR37 and SE by the second year. The percentage of samples infested with root aphid was consistently the lowest in AR37 pastures with significantly more infestations in AR1 and Nil pastures by 2008, when overall levels were the highest. Grass grub numbers also increased annually with no significant difference between treatments. The results are consistent with AR37 pastures having the lowest insect pressure and this was reflected in their superior ryegrass tiller density which was maintained during the 2008 drought.

Keywords: *Neotyphodium*, Argentine stem weevil, black beetle, root aphid, grass grub, pasture persistence

Introduction

Microscopic fungal endophytes (*Neotyphodium* spp.) live systemically within their grass hosts, have no external form and are transmitted via seed. They produce secondary metabolites in the form of alkaloids which can have detrimental effects on both mammalian and insect herbivores. In New Zealand the standard or wild-type endophytes in perennial ryegrass (*Lolium perenne*) produce three major alkaloids, peramine, ergovaline and lolitrem B but in the original habitats of ryegrass in Europe diverse strains of endophytes with different alkaloid profiles can be found (Easton *et al*. 2001). Some endophytes produce none of these common alkaloids. The differential effects of alkaloids on livestock and insects has had important implications for agriculture in New Zealand, allowing the development of endophyte associations that minimise or eliminate the undesirable effects of endophytes while retaining their desirable attributes (Easton *et al*. 2001).

In 2000, perennial ryegrass with the endophyte AR1 was commercially released to New Zealand farmers. This endophyte does not produce the mammalian toxins, lolitrem B or ergovaline, but does produce peramine, which protects its host from Argentine stem weevil (ASW) (*Listronotus bonariensis*) attack (Rowan *et al*. 1990) without any detrimental effects to livestock. AR1 has also been associated with reduced populations of another insect pest, pasture mealybug (*Balanococcus poae*) (Pennell *et al*. 2005) but has only a moderate effect on black beetle (*Heteronychus arator*) (Popay & Baltus 2001). Another endophyte, AR37, which produces a complex of epoxy-janthitrems but none of the common alkaloids (Tapper & Lane 2004), has been evaluated alongside AR1. This endophyte adversely affects ASW (Popay & Wyatt 1995) and black beetle (Ball *et al*. 1994; Hume *et al*. 2007) and reduces populations of pasture mealybug (Pennell *et al*. 2005). Subsequent studies have shown that it also virtually eliminates populations of a root aphid (*Aploneura lentisci*) (Popay *et al*. 2004; Popay & Gerard 2007; Hume *et al*. 2007) and reduces survival and feeding damage by porina (*Wiseana cervinata*) (Jensen & Popay 2004).

The broad spectrum insect control provided by AR37 has been associated with increased ryegrass yields in field experiments (Hume *et al*. 2004; 2007). However, experiments with sheep have also shown that AR37 can cause outbreaks of ryegrass staggers, although these tend to be less frequent, more ephemeral and often less severe than that caused by the toxic standard endophyte (Fletcher & Sutherland 2009). That study also showed that AR37 does not depress growth rates of lambs as found with the standard (wild-type) endophyte.

It is important that any new endophyte also be evaluated for effects on dairy cow health and milk production. In 2005, DairyNZ initiated a paddock-scale trial to compare the two new endophytes, AR1 and AR37, with the standard endophyte (SE) and Nil endophyte ryegrass for their effects on cow health, milk production and pasture production. As part of this trial, Argentine stem weevil damage and soil pest populations were determined annually between 2006 and 2009. This paper reports the first 3 years of these insect results.

Materials and Methods

A paddock-scale field trial was established at Scott Farm close to Hamilton on a Matangi silt loam soil. Existing pasture was sprayed out twice before cultivation and drilling on April 19, 2005. Perennial ryegrass cv. 'Commando' without endophyte (Nil) or infected with the fungal endophytes AR1, AR37 or the standard endophyte (SE) was sown at 18 kg/ha, each with white clover (*Trifolium repens*) cv. 'Tribute' sown at 3 kg/ha. Each treatment was replicated in six 0.5 ha paddocks randomly allocated to treatments. At sowing, grow-out tests confirmed high infection levels in the endophyte-infected treatments (86–98%) whereas the Nil treatment had no endophyte. The endophyte infection rates were then monitored annually in autumn by immunoblotting at least 50 tillers per replicate paddock.

Applications of P (47 kg/ha), S (77 kg/ha) and Mg (14 kg/ha) were made in autumn (March/April) to all paddocks with K (50 kg/ha) being applied in late spring/ early summer (September to December) of each year. Regular soil testing showed levels of P, K, S and Mg were adequate for unimpeded pasture growth. Nitrogen (37 kg/ha, as urea) was applied regularly from late winter to late spring and in autumn of each year. Total N applied annually (1 June to 31 May) ranged from 170 to 259 kg/ha. The experiment was rotationally grazed by dairy cows at a stocking rate of 3.0 cows/ha.

Argentine stem weevil adult feeding and larval damage were measured in mid January of each year from 2006 to 2008. Twenty clumps of tillers were taken randomly across each replicate paddock by cutting them below the crown and four tillers were sampled at random from each clump. Each of the 80 tillers per paddock was scored for adult feeding on the leaf blades using a scale from 0–5 where 0 = no feeding scars and 5 = a large number of feeding scars present. Tillers were then inspected for the presence of larval damage. Each tiller that was damaged by ASW larvae was scored for the severity of that damage on a scale of 1–3 where 1 = minor damage to the outside of the pseudostem without penetration of the tiller; 2 = penetration and a small amount of mining inside the tiller; and 3 = extensive mining of the tiller or destruction of the meristem by mining through the base of the tiller.

In late February/early March of each year a census of soil insects in each replicate paddock was carried out. Ten spade squares (200 × 200 mm to a depth of approximately 150 mm) were taken at random across each paddock and the soil sorted in the field. Numbers of black beetle, grass grub and clover root weevil were recorded and converted to a population density. The number of samples in which root aphid was present was also noted.

Data for the 3 years were analysed together by a general analysis of variance with no blocking and using year as a factor. Means were separated by calculating a least significant difference using Tukey's HSD test.

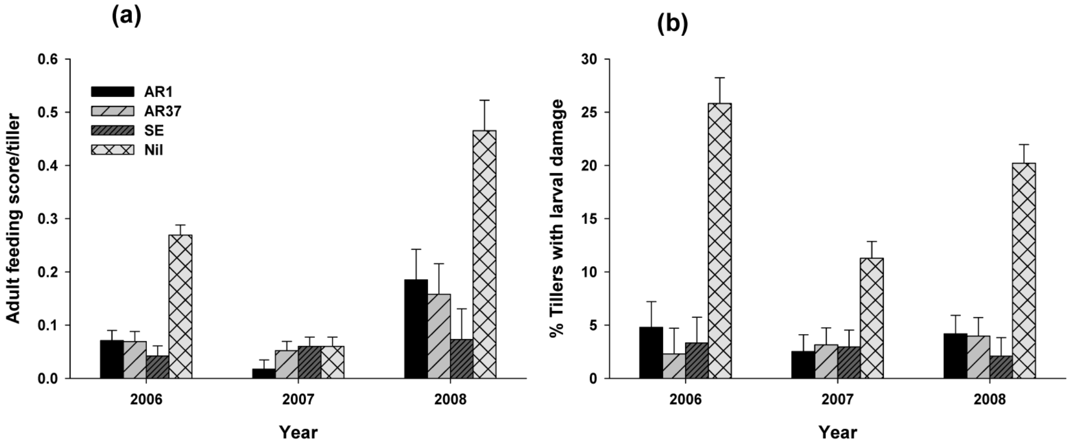
Results

Endophyte-infection levels in tillers taken from the Nil paddocks increased from 0% at sowing to 5% in 2006, 9% in 2007 and 11% in 2008. In the endophyte-infected treatments, endophyte levels remained at high levels for these 3 years, averaging 96±3%, 88±1% and 97±1%, respectively, for AR1, AR37 and SE.

Argentine stem weevil

In 2006 and 2008, more adult feeding was recorded on the Nil tillers than on any of the endophyte-infected treatments ($P<0.001$), but in 2007 adult feeding was low (<0.06/tiller) in all treatments (Fig. 1a). The percentage of tillers damaged by larvae was significantly ($P<0.002$) reduced by all endophytes compared with the Nil

Figure 1 (a) Argentine stem weevil adult feeding score/tiller and (b) percentage of tillers damaged by larvae on different endophyte treatments for each year between 2006 and 2008. Error bars represent the SEM.



treatment in each of the 3 years (Fig. 1b). In January 2006, an average of 25% of tillers in Nil paddocks were damaged by larvae, with a maximum of 40% of tillers damaged in one paddock. Larval damage in the endophyte treatments averaged less than 5% with a maximum of 6%.

All tillers that were damaged by ASW over the 3 years (n=98, 71, 68 and 268 for AR1, AR37, SE and Nil, respectively) were classed as having minor, moderate or severe damage. AR37 had a higher percentage of tillers with minor damage compared with Nil (49% cf. 27%; LSD 20.2; $P<0.05$) and conversely, fewer tillers that were severely damaged relative to those on Nil plants (33% cf. 56%; LSD 21.0; $P<0.05$). The percentage of tillers with minor and severe damage for SE (42% and 35%, respectively) and AR1 (36% and 40%) was not significantly different from either Nil or AR37.

Soil insects

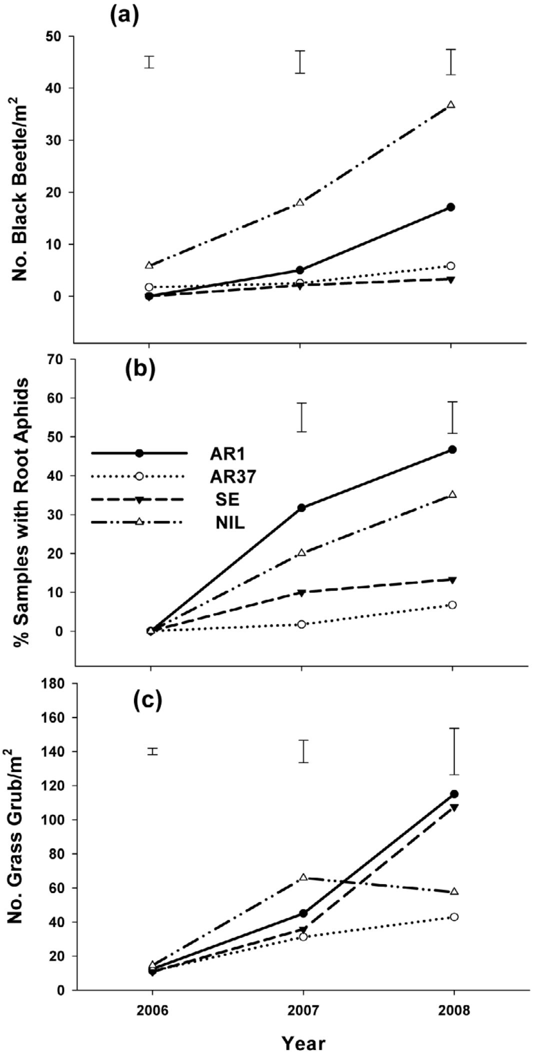
The major soil insect pests monitored were black beetle, grass grub (*Costelytra zealandica*), root aphids (*A. lentisci*) and clover root weevil (*Sitona lepidus*). White fringed weevil (*Naupactus leucoloma*) and Australian soldier fly (*Inopus rubriceps*) were also found frequently but data on these are not presented.

At the time of sampling, black beetle were found as larvae, pre-pupae, pupae and adults. In 2006, average populations were low (<10/m^2) with no significant differences among the treatments (Fig. 2a). The maximum number recorded in any one paddock in 2006 was 28/m^2 in a Nil treatment. Populations had increased substantially (on average over a four-fold increase) in the Nil paddocks by late summer 2007 but remained low in all the endophyte-infected treatments, resulting in a significant difference between these and the Nil paddocks ($P=0.053$). Populations varied considerably among different paddocks, ranging from 0 to 43/m^2 in the Nil paddocks but only between 0 and 13/m^2 in the endophyte-infected paddocks. Between 2007 and 2008 there was a further increase in black beetle populations in both the Nil and AR1 treatments, while numbers under AR37 and SE remained low. Nil paddocks had significantly higher black beetle populations than both AR37 and SE ($P<0.001$) while AR1 populations were not significantly different from the other treatments.

Root aphid was not recorded in any of the samples in the first year but in 2007 it was found in 32% of the samples taken in AR1 paddocks and 20% taken in Nil paddocks (Fig. 2b). Only 2% of samples in AR37 contained root aphid, fewer than that in AR1 ($P=0.052$) but not significantly different from Nil or SE. Over all treatments there was a significant increase in the frequency of samples containing root aphid from an average of 16% in 2007 to 26% in 2008 (LSD 9.0, $P<0.001$). In 2008, AR37 had fewer samples containing root aphid than both AR1 and Nil treatments ($P<0.001$). In addition SE also had fewer samples infested than AR1 ($P<0.05$) but the difference between SE and Nil was not significant.

Grass grub densities increased significantly each year between 2006 and 2008 (average populations in 2006, 2007 and 2008, respectively: 12, 45, and 81/m^2, LSD 24.7, $P<0.001$). Nil paddocks did not fit with this pattern between 2007 and 2008 when average

Figure 2 (a) Density of black beetle (larvae, pre-pupae, pupae, and adults) populations (b) percentage of samples infested with root aphid and (c) density of grass grub populations measured annually between 2006 and 2008 in pastures with different endophyte treatments. Error bars represent the SEM.



population levels declined in these paddocks (Fig. 2c). AR37 consistently had lower populations than other treatments but variability among the replicate paddocks meant that the differences were not significant.

Clover root weevil was present, mainly as larvae, but with some pupae and adults when sampling was carried out each year. Populations were highest in 2006, averaging 84/m^2 but were at relatively low levels in 2007 (12/m^2) and 2008 (25/m^2). There were no differences between treatments.

Discussion

This trial has provided a rare opportunity to gather extensive data on the effect of different endophytes on a range of insect pests over a period of time at the paddock scale. These data confirm information from pot experiments and small plot field trials (Popay *et al*. 1999; Popay & Baltus 2001; Popay & Gerard 2007; Hume *et al*. 2007) showing that the endophytes AR1, AR37 and SE are equally effective at suppressing damage in perennial ryegrass by ASW but that they have differential effects on two other insect pests, black beetle and root aphid. For black beetle, AR37 provides the same level of control as the standard endophyte whereas AR1 has only a moderate influence on this pest. AR37 has also shown strong resistance to root aphid in this trial, with a low percentage of samples infested throughout compared with the Nil and AR1 treatments but similar levels to SE. Previous studies have indicated that SE (also called the wild-type endophyte) can suppress root aphid populations but these effects tend to be transitory with other measurements showing that populations are similar to those on Nil ryegrass (Popay *et al*. 2004; Popay & Gerard 2007). It is likely that seasonal environmental conditions affect the level of resistance conferred by the SE treatment whereas AR37 maintains a strong resistance to root aphid throughout the year.

The current trial has also illustrated the patterns of invasion by different insect pests into new pastures following cultivation. ASW and clover root weevil were quick to colonise the establishing pastures within a few months of sowing whereas populations of black beetle, grass grub and root aphids increased from low levels in 2006 to become significant problems in susceptible pastures by 2008. An exception to the overall increase in grass grub numbers occurred in Nil paddocks between 2007 and 2008 and this is attributed to an interaction with the increased number of black beetle in these treatments over that period (Fig. 2a cf. Fig. 2c). The damage caused by black beetle larvae feeding on roots of grasses in January and February is likely to have severely depleted the food resources for the smaller grass grub at that time. Damage by ASW larvae in the Nil pastures was low in 2007 but increased again in 2008 whereas population densities of clover root weevil were much lower in 2007 and 2008 than in 2006. The densities of clover root weevil recorded in March 2006 were probably insufficient to have caused major damage to the clover, but their populations tend to peak during their winter generation when they are most likely to reduce clover productivity (Gerard *et al*. 2007).

Endophyte-mediated resistance to the ryegrass pests ASW, black beetle and root aphid has been associated with increased yields of ryegrass in field experiments (Popay *et al*. 1999; Hume *et al*. 2007). In the first 2 years of pasture monitoring of this trial, Thom *et al*. (2008) found that annual and seasonal production was similar across all treatments. By May 2007 ryegrass tillers densities were slightly higher ($P<0.10$) in AR37 (6040 tillers/m^2) than in SE (5246), AR1 (4574) and Nil (4192) (Thom *et al*. 2008). In spring 2007, ryegrass content of AR37 pasture (85% of DM) was higher than for AR1 (74%) or Nil (78%) with a concomitant lower clover content. SE pastures were similar to AR37. Ryegrass tiller densities in March 2008 during that summer's drought were higher ($P<0.001$) in AR37 pastures (3420 tillers/m^2) than in SE (1930), AR1 (1680) and Nil (810) (Thom 2008).

The severe drought in the Waikato in 2008, in combination with the insect pressure, took its toll on the pastures. All the Nil treatments were abandoned and re-sown with AR1, AR37 or SE in autumn 2008. Endophyte-infected pastures with fewer than 2000 tillers/m^2 were undersown with the same treatment. This necessitated undersowing three of the AR1 and SE paddocks but only one of the AR37 paddocks indicating the better persistence of ryegrass infected with AR37 under adverse conditions. The gradual decline in tiller numbers and the eventual failure of the Nil pastures could be attributed to the combined effects of ASW, black beetle and root aphid together with the effects of the drought. However, the reasons for the relative differences in tiller densities in the SE and AR1 paddocks compared with AR37 by May 2007 and the need to undersow half of these paddocks in 2008, are hard to pinpoint. Black beetle populations under AR1 were not considered to be at damaging levels by 2008, with the exception of one paddock with a density of 45/m^2. Similarly, SE had uniformly low populations of black beetle over the 3 years of monitoring. Neither AR1 nor SE was damaged by ASW. There is a strong possibility, therefore, that root aphid infestations have contributed to the apparent decline in tiller density and persistence of the ryegrass in these pastures, despite the low level infestations recorded on SE. As indicated above, root aphid populations under SE are likely to be variable throughout the year. Decreases in ryegrass productivity in laboratory pot trials (Popay & Gerard 2007) and in field plots (Hume *et al*. 2007) have been attributed to infestations by this aphid. Grass grub may also have contributed to the total pest pressure on the pastures, particularly in 2008 when populations over 200/m^2 were recorded in some AR1 and SE paddocks, while the highest population under AR37 was 108/m^2.

Poor pasture persistence is a serious issue for New Zealand farmers which has been exemplified in this experiment. Within 2 years of establishment, differences in ryegrass tiller densities were becoming apparent (Thom *et al*. 2008) and this was exacerbated during the severe drought in 2008 to the point where several pastures needed renovation. The most badly affected were the Nil pastures, suggesting that insect pest pressure, in combination with the drought, was a major contributing factor to the demise of these pastures. Other evidence supporting this interpretation is that AR37 pastures, with the lowest overall insect pressure from ASW, black beetle, root aphid and grass grub, had the highest tiller densities and survived the drought better than SE and AR1.

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