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Argentine stem weevil: farmer awareness and the effectiveness of different ryegrass/endophyte associations

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Abstract
Argentine stem weevil (ASW) is a highly destructive pest of ryegrass that has recently been associated with increased incidences of field damage. A survey of farmer awareness and management practices in relation to this pest was carried out. Many (47%) farmers sowed favoured plant hosts such as short-term and tetraploid ryegrasses. A field trial, undertaken near Hamilton, compared the effects on ASW larval damage of 10 different ryegrass/endophyte associations in comparison with an endophyte-free control with and without seed treatment. U2 endophyte in a diploid perennial festulolium and AR37 endophyte in a hybrid tetraploid, an Italian diploid and a tetraploid perennial ryegrass had significantly less damage (<26%) than all other treatments (>42%). NEA2 endophyte in a diploid perennial ryegrass and AR1 endophyte in short-term ryegrasses failed to protect plants from severe damage by this pest. Farmers need to be aware of the risks of ASW damage to short-term and tetraploid ryegrasses.

Keywords: ploidy, short-term ryegrasses, larval damage, endophyte strains, pasture management

Introduction
Argentine stem weevil (ASW) (*Listronotus bonariensis*) is one of New Zealand's most destructive grass and cereal pest. First reported in the country in 1927, this small cryptic insect occurs throughout New Zealand. Adult weevils feed on the emergent cotyledons of newly sown grasses and cereals, killing young seedlings (Goldson *et al.* 1998). The larval stages mine inside tillers, often killing the meristem and causing widespread tiller death in susceptible hosts. Between September and March ASW undergoes at least two generations in most areas except where cool temperatures (e.g. southern South Island) may slow its development allowing only one generation per year (Barker *et al.* 1989; Goldson *et al.* 1998). Damage is particularly apparent in the summer/autumn, but is often wrongly attributed to drought (Whatman 1959).

An important discovery in the early 1980s showed that a seed-transmitted fungal endophyte (*Epichloë festucae* var. *lolii*; formerly *Neotyphodium lolii*) infecting perennial ryegrass (*Lolium perenne*), which had become naturalised in New Zealand, provided its host with resistance to ASW (Prestidge *et al.* 1982; Mortimer & di Menna 1983). To exploit endophytes for control of this insect, however, it was necessary to source endophyte strains that minimised or eliminated the mammalian toxicity caused by the naturalised common toxic strains. New strains were introduced that did not produce the two toxins ergovaline and lolitrem B or produced them at low concentrations. In 2001, AR1, an endophyte producing the ASW deterrent, peramine, but not the toxic alkaloids was commercialised (Thom *et al.* 2012). This was followed in 2007 by the release of AR37 which produces epoxy-janthitrems but not peramine, ergovaline or lolitrem B. Since then seed companies have commercialised their own *E. festucae* var. *lolii* endophyte strains such as NEA2, that rely on low production of peramine and the toxic alkaloids and an *E. uncinata* endophyte, U2, isolated from meadow fescue, which produces loline alkaloids. U2 is now available in a *Festulolium* cv. 'Barrier' which has many of the characteristics of its meadow fescue parent.

A parasitoid (*Microctonus hyperodae)*, that sterilises adult ASW, was imported and released in the early 1990s to provide additional control of this pest, particularly in susceptible plants not protected by endophyte. Popay *et al.* (2011) reported on incidences of ASW damage in the field, concluding that parasitism may no longer be suppressing populations. Recently, a decline in parasitism levels has been confirmed (Goldson *et al.* 2014; Tomasetto *et al.* 2017). Although the reasons for this decline have yet to be identified, the situation has led to concerns about farmers' current awareness of ASW and their decision-making with regard to managing it. Seedling ryegrass and cereals that are susceptible to adult and larval damage at establishment need to be protected by seed treatment. In addition, susceptibility to ASW is higher in Italian and annual species (*Lolium multiflorum*) and tetraploid perennial ryegrasses compared with diploid perennials (Goldson 1982; Barker 1989; Prestidge 1991; Popay *et al.* 1995) that may not always be overcome by endophyte-infection (Popay *et al.* 2003). This paper reports on a survey seeking to gauge farmers' level of knowledge

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of ASW, as well as to understand what governs their decision making on planting, establishment methods and use of endophytes and seed treatments for grasses and cereals. With the current availability of endophytes in short-term ryegrasses and a festulolium, it was timely to also investigate ASW larval damage in a range of ryegrass/endophyte combinations in a field trial. The information from the survey and the field trial will lead to a set of recommendations on how to reduce the impact of ASW.

Methods

Farmer survey

In autumn 2015, a digital survey was planned to gain insights into the decision making process of farmers and growers in relation to insect pest management in general and ASW in particular. The survey had a sample size of 500 different farmers and growers from dairy, sheep and beef, and arable sectors with an equal distribution across the main regions of New Zealand, resulting in a stratified random sample of the total population. The contact details were obtained from a database owned by AssureQuality. Unfortunately, technical difficulties impacted on already tight timeframes and a digital survey was no longer deemed feasible. Instead, a telephone survey was undertaken, using the same questions as would have been used in the digital survey. A sample of 2000 telephone numbers was created using AssureQuality's database, with the same requirements of an equal distribution of the sample size among the different regions of New Zealand, and only including arable, dairy, and sheep and beef farmers (e.g. farm owners, managers, sharemilkers, and others on-farm). Given the tight timeframes, it was not possible to allow the survey to run over an extended period and collect as much data as possible, instead the survey was finished as soon as there were 100 completed questionnaires. This means that the margin of error is 10%, based on a 95% confidence interval (Sciencebuddies 2015). The questions covered five areas: 1. Personal information; 2. Sowing practices; 3. Knowledge of insect pests; 4. Awareness of ASW; 5. Sources of information. Due to the timing of the survey (i.e. before the 2015 sowing was completed), the questions regarding sowing practices focused on the year 2014. The data were analysed in Excel.

Field Trial

A field trial was established on a Motumaoho shallow silty peat soil in two adjoining paddocks at AgResearch's Ruakura Research Centre farm, Hamilton, Waikato (GPS coordinates: 37°45'60.0"S 175°19'21.6"E60) in May 2015. Existing perennial ryegrass/white clover pasture was sprayed out with herbicide before direct-drilling 10 cultivar/endophyte associations, using untreated seed (Table 1). An endophyte-free (Nil) treatment was sown with untreated seed and with seed treated with Ultrastrike to give 12 treatments in total (Table 1). Plots were 10 x 1.5 m in a randomised block design with four blocks; two blocks were in one paddock and the other two in the adjacent paddock. Seed was direct-drilled at a rate of 26 kg/ha for tetraploid ryegrass and 20 kg/ha for the diploid grasses. Annual tetraploid ryegrass without endophyte (cv. 'Winter Star II') was sown around the outside of the plots to ensure there was high ASW pressure within the trial. The area was rotationally grazed by cattle to control herbage mass and was not irrigated. It received a maintenance fertiliser application of superphosphate at 250 kg/ha and selenium (Selprill) at 1 kg/ha in late September 2015.

Table 1 Percentage of tillers infected with endophyte, and percentage of tillers (±SE) with any level of Argentine stem weevil larval damage (LD) or with moderate and severe damage only for different ryegrass/endophyte combinations and a Nil control.

Ryegrass types[1]	Ploidy	Cultivar	Endophyte	% inf.[4]	% LD (±SE)[2]	%LD Mod & Sev[2]
Short	Tetra	'Delish'	AR1	94	42.6 (5.1)	26.6 (4.6)
Long	Tetra	'Ohau'	AR1	95	45.3 (5.1)	28.4 (4.6)
Long	Tetra	'Ohau'	AR37	93	22.6 (4.3)	8.6 (2.9)
Long	Tetra	'Banquet II'	Endo5	84	56.0 (5.4)	39.3 (5.3)
Italian	Tetra	'Feast II'	Nil	16	56.0 (5.4)	35.7 (5.2)
Italian	Tetra	'Feast II' ST[3]	Nil	13	42.5 (5.3)	27.6 (4.8)
Italian	Diploid	'Asset'	AR37	98	26.5 (4.5)	15.3 (3.6)
Perennial	Tetra	'Halo'	AR1	80	43.8 (5.5)	30.0 (5.1)
Perennial	Tetra	'Halo'	AR37	97	19.6 (4.0)	16.5 (3.8)
Perennial	Tetra	'Bealey'	NEA2	85	42.4 (5.4)	22.4 (4.5)
Perennial	Diploid	'Trojan'	NEA2	83	50.6 (5.5)	30.1 (5.0)
Perennial	Diploid	'BarrierCombo'	U2	83	20.5 (4.4)	2.4 (1.7)

[1] Short and long rotation: *Lolium boucheanum* syn. *L. hybridum*; Italian: *L. multiflorum;* perennial: *L. perenne.*
[2] Percentage of tillers with larval damage based on tillers with the appropriate endophyte status.
[3] Seed treated with Ultrastrike. [4]Percent of tillers infected with endophyte.

On 2[nd] February 2016, all plots were sampled to determine the extent of ASW damage. In each plot, 25 small clumps of tillers were cut below ground level and from each clump one live tiller was selected at random for examination for ASW damage. The number of adult feeding scars on leaf blades was counted for each tiller, taking scar size into account, and the number of ASW eggs was recorded. Each tiller was immunoblotted for determination of its endophyte status (Simpson *et al.* 2014). It was then examined under magnification, if necessary, for ASW larval damage. Damage was classed as minor where there was scarring on the outside of the tiller, moderate where the larvae had penetrated and partially mined the centre of the tiller and severe where the tiller was extensively mined or a hole had been bored directly through the base in the region of the meristem. Dead tillers were also removed from the clumps and checked for larval damage.

Only data from live tillers with the expected endophyte status (positive for endophyte-infected, or endophyte-free in 'Nil' treatments) were analysed. Adult feeding and oviposition were analysed by ANOVA in Genstat v 16 using replicate as the blocking factor. Each of the larval damage variables was compared between treatments using a generalised linear model, assuming treatment-group-specific binomial distributions through logit link function. These analyses were carried out using Minitab version 16. The host/endophyte combinations used in the trial did not allow analyses for effects of ploidy and ryegrass species to be undertaken.

Results

Farm survey

There was an over representation of sheep and beef farmers in the sample of 100 farmers that were surveyed, with 77% in this category although 22 of these farmers also had involvement in other sectors, for example, dairy support or cropping. A total of 13 respondents were dairy farmers. The majority of the respondents were over 55 and male. They predominantly had co-ownership of the farm, with more than 15 years farming experience, and the majority came from Canterbury (n=18), Manawatu-Whanganui (n=16), Otago (n=14) or the Waikato (n=13) regions. The results hence show a bias in the results towards South Island sheep and beef farmers; nonetheless we consider the answers relevant for a large proportion of the farmer population in New Zealand, given that the answers of the other 23 farmers were largely in line with the 77 sheep and beef farmers.

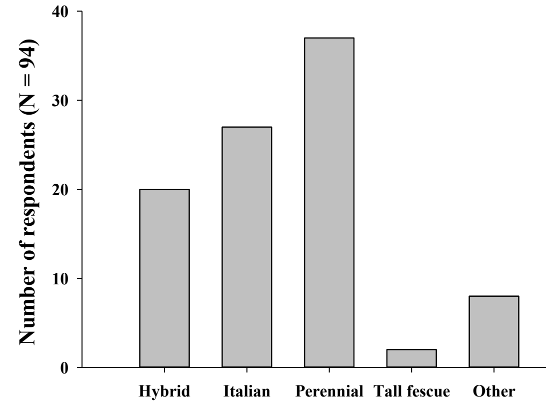

Figure 1 Ryegrass species (Hybrid: *Lolium boucheanum* syn. *L. hybridum*; Italian: *L. multiflorum*; perennial: *L. perenne*) sown by farmers in 2014, as reported from a survey of 100 farmers carried out in autumn 2015.

Twenty respondents had sown cereals in 2014, of which six sowed two or three different types of cereals, hence there were 28 occasions on which cereals were sown, of which 41% were barley, and 18% wheat. A total of 62 respondents sowed grasses in 2014, of whom 26 had sown more than one type of grass, resulting in 94 reports of establishment of new grasses. The majority (n=47) had used short-term ryegrasses (either short or long rotation/hybrids (*Lolium boucheanum* syn. *Lolium. hybridum*) (n=20) or Italians/annuals (n= 27), and 37 had sown perennial ryegrass (Figure 1). Respondents in the category 'other' used clover, or clover and ryegrass, but did not indicate what type of ryegrass. Most (n=30) reported using a diploid and the remainder (n=22) a tetraploid, except for Italian and annual ryegrasses where tetraploids were favoured over diploids. Interestingly, about 39% of all responses were in the 'I don't know' category.

Regarding choice of endophyte, 29 of 94 responses used AR37 while another 27 did not know which endophyte they used. Endophytes such as NEA2, Endo5, U2 and MaxP™ were not selected, although these were options where relevant. When asked what determined their choice of endophyte the majority (53% of respondents) said previous experience was a major factor.

Of the 20 respondents who had sown cereals, 80% used insecticide-treated seed, whereas only 61% of farmers who had sown ryegrass used treated seed and 19% reported not using any seed treatment. The majority of farmers who had sown either cereals or grasses were either very satisfied (50%) or satisfied (40%) with establishment.

Insect pests were regarded by most respondents as being very important or important factors during establishment of pastures and cereals (69%) as well as for productivity (75%) and persistence (73%). Hence the majority also regarded the use of seed treatment (84%) and selection of endophyte (68%) to be important or very important. Despite this, 50 of the 65 respondents who had sown grasses or cereals reported that they were unaware of any insect pest damage during the establishment phase. The remaining 15 thought more than one insect pest was a problem, with only two reporting ASW and/or clover root weevil as a cause of damage during establishment. Those who had sown cereals or grasses the previous year tended to be more confident in recognising insect damage.

When specifically asked about ASW, 92% of respondents had heard of this insect, but only 25% considered it to be important. This lack of concern was not necessarily based on a well informed decision. Four respondents were not concerned about ASW because they were well informed about insect pests on their farm, for example, through a farm consultant. On the other hand, three respondents gave lack of information as a reason for not being concerned, saying "I don't really know what I'm looking for. I rely on the fact that others say, that by far the most important in this area is grass grub and black beetle." or "I haven't been educated in recognising it." Moreover, most of those who did not consider ASW to be important on their farm attributed this to their location (e.g. southern parts of New Zealand or steep hill country). Of those 23 respondents that were concerned about ASW, at least one respondent had mistaken it for clover root weevil saying "It just devastates all the clover." Figure 2 shows that the confidence levels in recognising ASW damage varied widely; 30% said that they were somewhat confident, 27% were confident, and 24% were not confident. These results in combination with the likelihood of others confusing ASW and clover root weevil indicate that the respondents overall were not particularly aware about ASW and its damage.

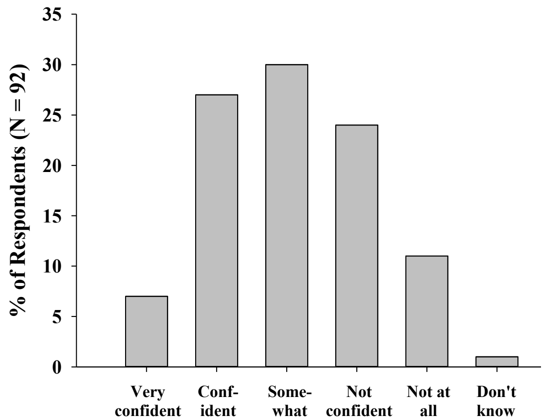

Figure 2 Farmer confidence in their ability to recognise Argentine stem weevil damage.

Field trial

Endophyte-infected treatments had high rates of infection (>80%) while the Nil treatments cv. 'Feast II' contained low levels of endophyte (Table 1). All results presented on ASW damage in Table 1 pertain only to those tillers with the expected endophyte status. Adult feeding was significantly higher on the two 'Feast II' Nil treatments (Mean: 5.1 and 3.6 feeding scars/tiller for untreated and treated seed, respectively) than for all infected treatments (<1.2 scars/tiller) (SE 0.36; df 33, P<0.001). Feeding was significantly lower in 'Feast II' sown with treated seed compared with the untreated seed (P<0.05). There were no significant differences in the amount of adult feeding among the endophyte-infected treatments which ranged from 0.56 scars/tiller for AR1 in 'Halo' to 1.2 scars/tiller for NEA2 in 'Bealey'. Egg numbers on tillers sampled from each plot ranged from 0.25 in 'BarrierCombo' with U2 to 6.25 in each of the Nil and Ohau treatments, but these differences were not significant (P>0.05).

Over 50% of tillers in some treatments showed symptoms of larval damage including those with minor scarring on the outer leaf sheath (Table 1). U2 in a diploid perennial ('BarrierCombo') and AR37 in association with a hybrid tetraploid ('Ohau'), an Italian diploid ('Asset') and a tetraploid perennial ('Halo') had significantly less damage (P<0.01) than all other treatments. When only those tillers with moderate or severe damage (i.e. serious damage) in which larvae had penetrated the tiller and at least partially mined it were considered, the percentage of damaged tillers in 'BarrierCombo' U2 dropped from 20% with some level of damage to 2% with serious damage (Table 1). This treatment had fewer tillers (P<0.01) with this serious level of damage compared with all other treatments except the hybrid 'Ohau' with AR37. There was no difference between the three AR37 treatments. These three treatments had less moderate or severe damage than all other treatments (P<0.05) except for a tetraploid perennial ('Bealey') with NEA2 which was not significantly different from 'Halo' and 'Asset' infected with AR37. 'Bealey NEA2' had less damage than 'Banquet II Endo5' (P=0.018), but all other treatments suffered similar levels of moderate and severe damage.

Across all treatments 404 dead tillers were checked for larval damage with the highest mean number per plot (±SE) occurring in the tetraploid Italian 'Nil' treatment, 'Feast II' (n=12.8±1.6), and the lowest number in 'BarrierCombo U2' (n=5.5±1.0) but this difference was not significant. The endophytes U2 in 'BarrierCombo', the three AR37 associations, AR1 in short rotation tetraploid 'Delish' and NEA2 in 'Bealey' and 'Trojan' all significantly reduced the proportion of dead tillers with larval damage compared with Nil. Overall the percentage of dead tillers that were damaged by larvae was 69, ranging from 40% in 'BarrierCombo U2' to 85% in 'Feast Nil'. Dead tillers could not be checked for presence of endophyte.

Discussion

Several interesting points emerged from the farmer survey. Given a national average annual rate of renewal of pastures in New Zealand of 3-4% (Pasture Renewal Charitable Trust: http://www.pasturerenewal.org.nz/), the proportion of respondents in the survey who had sown new pastures in the previous year (62%) was high. Many of these were using the short-term grasses known to be particularly vulnerable to ASW damage. Use of seed treatment to protect young grass and cereal seedlings at establishment was common, although some chose not to, thereby running the risk of insect damage. The vast majority of farmers used endophyte, with endophyte choice based on experience, although many did not know which endophyte they had used. Most reported a high level of satisfaction with establishment of their new sowings.

Although 92 farmers had heard of ASW before, many farmers thought this insect was not posing a risk to them due mainly to their farm location. The main regions the survey respondents were located in (Waikato, Manawatu-Whanganui, Canterbury, Otago), however, are all likely to be vulnerable to ASW, with the possible exception of Otago. Cool temperatures in this and the Southland region were thought to restrict the number of generations that ASW could complete in the summer to one (Ferguson *et al.* 1994), but recent anecdotal evidence now suggests that two generations are more likely the norm (C.M. Ferguson pers. comm.). Many sheep and beef farmers, however, may be farming in cooler hill country areas where there is a perceived lack of risk. Nevertheless this result, and the other responses from the survey, also suggests that farmers do not recognise the insidious damage caused by this insect which often coincides with drought.

The extensive use of short-term grasses and tetraploids is a concern in the light of the high levels of damage that occurred in the field trial. Moreover, these damage levels are likely to have been underestimated as they have not taken full account of the more extensive damage in the dead tillers that could not be checked for endophyte. AR1, which gives strong protection to a diploid perennial ryegrass host (Popay *et al.* 1999; Thom *et al.* 2014) did not significantly reduce the amount of moderate and severe larval damage when infecting a short rotation ryegrass, a hybrid, or a tetraploid perennial host compared to an endophyte-free annual. This is likely to be due to reduced peramine concentrations in combination with the weevil's greater preference for these ryegrasses compared with diploid perennials (Popay *et al.* 2003). On the other hand, AR37 in similar hosts significantly reduced damage compared with both AR1 and Nil. NEA2 even in the diploid perennial, did not provide strong protection. The strongest effect on ASW, however, was exerted by the U2 endophyte which produces loline alkaloids that are known to reduce both ASW egg laying and larval damage (Jensen *et al.* 2009; Popay *et al.* 2009).

Although seed treatment is aimed at protecting seedlings during the vulnerable establishment phase, its benefits may persist in the longer term. As has occurred in other field trials (Popay & Thom 2009), adult feeding in AR37 plots in the field trial was significantly less than Nil despite this endophyte having no effect on adult ASW (Popay & Wyatt 1995). This almost certainly reflects a lower population of ASW in this treatment. Similarly, and unexpectedly, the seed treated Nil had significantly less adult feeding than untreated Nil. This suggests there has been some carryover effect of seed treatment via reduced populations of the adult weevil. Such an effect perhaps deserves more attention, even though in this case it did not result in significantly less larval damage.

Rates of parasitism of ASW in the annual tetraploid ryegrass surrounding the field plots did not exceed 14% near the end of December (P.J. Gerard pers. comm.) when a new generation of ASW was laying eggs. These parasitism levels are unlikely to have had any major impact on reducing the amount of oviposition and as a result, significant levels of damage occurred. This reinforces the need for farmers to continue to be vigilant with regard to ASW. Their decisions regarding choice of ryegrass/endophyte combinations, and use of seed treatment, are critical if they want to avoid ASW larval damage. A greater level of knowledge and understanding of these issues would assist farmers in making those decisions.

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Lime application can help protect pastures against black beetle

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Abstract

Black beetle attacks pasture grasses in the northern and coastal North Island and with a warming climate, the beetle has extended its range and damage has become more prevalent. On-farm investigations into prevention of damaging populations suggested that black beetle density was inversely related to soil pH. Two replicated block experiments, the first in 2013-2015 on two farms, and the second on four farms in 2015-2017 investigated the effects of late spring agricultural lime applications at the rate of 5 tonne/ha on summer black beetle populations. The results showed that lime can help suppress black beetle populations. Importantly, the effect of lime persisted into the second year in Trial 2, preventing larval populations reaching damaging levels of over 40/m^2. This adds to the already well-known benefits of lime in improving soil health and pasture quality, vigour and persistence.

Keywords: agricultural lime, soil pH

Introduction

Black beetle (*Heteronychus arator* (Fabricius) Coleoptera: Scarabaeidae) is a subtropical pest of pasture grasses in New Zealand districts with a mean annual surface air temperature of 12.8ºC and above (Watson 1979). This includes Northland, Waikato, Bay of Plenty and coastal areas of the northern North Island from Whanganui in the west, around to Cape Kidnappers in the east (Bell *et al.* 2011). With the warmest (2016) and 3rd warmest (2013) years in the last decade and above average seasonal temperatures common (NIWA 2017), damage has become more prevalent in traditionally sporadically infested areas, and the beetle has extended its range inland and southward with sports field turf damage reported in Foxton in 2014 (B. Hannan pers. comm.).

Important factors contributing to damaging black beetle populations are above average temperatures, free-draining soils and availability of favourable food resources. Widespread black beetle outbreaks are associated with strong La Niña weather patterns which, on average, bring warmer than normal temperatures over the North Island in spring and autumn (Gerard *et al.* 2013). High spring temperatures (growing degree days above 15ºC, King *et al.* 1981b) encourage population increase while wet conditions are unfavourable for early instar larval survival (King *et al.* 1981c). C_4 grasses, as well as ryegrasses (*Lolium* spp.) without a deterrent endophyte, are favourable hosts (King *et al.* 1981a; Blank & Olson 1988; Ball & Prestidge 1992). In contrast, adult feeding, and in turn survival and oviposition are reduced by ryegrasses containing standard, AR37, NEA2 or Endo 5 endophytes (Ball *et al.* 1997; Popay & Baltus 2001; Bell *et al.* 2011).

A major outbreak of black beetle occurred in Waikato and Bay of Plenty from 2007 to 2010, and many farmers experienced widespread failure of perennial pastures (Bell *et al.* 2011). While climate and black beetle were not the only factors, losses were reported of about $1300/ha/year during this period (Reynolds 2013). Since then, the widespread use of black beetle-active ryegrass endophytes, in particular AR37, has enabled pastures to persist even under drought conditions (Thom *et al.* 2014). However, with ongoing higher annual temperatures, pastures on peat or light soils still experience damage. Consequently there remains a high demand for additional practical tools to help combat this pest in established pastures.

In the course of analysing data gathered from 12 paddocks across five Waikato farms during a black beetle/pasture persistence study, it was found that black beetle density was inversely related to soil pH (Gerard *et al.* 2013). This paper reports on field studies undertaken to investigate if the application of agricultural lime can help reduce black beetle populations.

Methods

Trial 1: 2013-2015

The trial was a randomised block design consisting of four paddocks on Waikato dairy farms, two on a farm with peat soils (Taupiri 1 and 2, 37°37'03.8"S 175°17'30.9"E) and two on a farm with ash soils (Waihou 1 and 2, 37°32'01.5"S 175°38'50.0"E). Each paddock (block) was divided into eight plots and AgLime supplied by McDonalds Lime (now Graymont) was applied at the rate of 5 tonne/ha to four randomly selected plots in each paddock on 4 November 2013, by the commercial operator, Wealleans, using a purpose built 4×4 ground-spreading vehicle. The timing of the lime application was during the black beetle oviposition period.

Black beetle populations were sampled by taking five 20 ×20 cm spade squares of turf to a depth of 15 cm and hand sorting in the field. This was done in December (a month after lime application) when the black beetle

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