AR128 - a new *Epichloë* fungal endophyte product for ryegrass

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Abstract

Perennial ryegrass (*Lolium perenne*) persistence and yield in many parts of New Zealand is critically reliant on its association with mutualistic fungal endophytes of the genus *Epichloë*. *Epichloë* spp. can protect their host plants from insect pests and improve tolerance to drought. Over the last 25 years several novel endophyte strains have been commercialised. While ensuring effective persistence of ryegrass, they have also minimised the negative impacts on livestock of ryegrass staggers, heat stress and productivity losses associated with the standard endophyte strain. A new endophyte product is becoming available – AR128. This *Epichloë* sp. LpTG-3 strain originated from Italy and has a similar known chemical profile to AR37, through expression of epoxyjanthitrems. AR128 protects the host ryegrass plant against the same insect pests as AR37. It also exhibits similar animal safety to that of AR37. However, AR128 transmits through seed production cycles at a greater frequency than AR37 and it also stores for a longer duration in seed at ambient temperature and humidity conditions. These features ensure that the end-user farmer obtains a high quality and effective product, which should ensure improved perennial ryegrass persistence and production compared to endophyte-free grasses and possibly other endophyte strains which are less amenable to transmission and storage. The human consumer of products derived from animals feeding on ryegrasses with AR128 can be assured that there are no food safety issues associated with this technology based on mouse feeding studies.

Keywords: animal welfare, endophyte storage, endophyte transmission, epoxyjanthitrems, insect pests

Introduction

*Epichlo*ë fungal endophytes are essential for perennial ryegrass (*Lolium perenne*) persistence in many regions of New Zealand (Johnson et al. 2013; Caradus 2023; Card et al. 2024) due largely to the combined impacts of insect pests, drought (Hewitt et al. 2021) and grazing pressure. Asexual *Epichloë* spp. are obligate mutualists (Caradus et al. 2021a) that produce a range of secondary metabolites some of which have a deterrent or toxic effect on invertebrates and vertebrates (Caradus & Johnson 2020; Johnson et al. 2021). There are four well characterised classes of alkaloids associated with *Epichloë* spp.: (1) 1-aminopyrrolizidines which include lolines; (2) pyrrolopyrazines which include peramine; (3) ergot alkaloids which include ergovaline, and (4) indole-diterpenes which include the tremorgenic compound lolitrem B (Schardl et al. 2012; Realini et al. 2024). The development of selected endophytes for pastoral agriculture has aimed at delivering strains which can protect ryegrass plants from insect pests with low impacts on ruminant animal health, welfare and productivity (Caradus et al. 2021b).

The common toxic or standard endophyte (SE) strain has been found throughout New Zealand and is marred by producing lolitrem B and ergovaline (Card et al. 2024). Lolitrem B is responsible for producing ryegrass staggers (Fletcher 2005; di Menna et al. 2012) and ergovaline for exacerbating heat stress (Klotz 2015;

ISSN 2463-2872 (Print) ISSN 2463-2880 (Online)

https://doi.org/10.33584/rps.18.2025.3771

Caradus et al. 2020), resulting in reduced milk and meat production by grazing animals (Fletcher 1999; Thom et al. 2012). The ryegrass endophyte strain AR1, released in 2001 (Easton et al. 2001), produces neither lolitrem B nor ergovaline (Fletcher 1999) but does produce a mammalian-safe compound, peramine, which controls adults of the introduced insect pest Argentine stem weevil (*Listronotus bonariensis*) (Popay et al. 1999; Ruppert et al. 2017). The uptake of AR1 by farmers in New Zealand was rapid, such that by 2008 about 70% of all proprietary perennial ryegrass seed sold contained AR1 (Caradus et al. 2013). However, it soon became apparent that a range of other insect pests were further affecting ryegrass persistence. So, while AR1 removed animal health, welfare and productivity issues it was not providing full protection against insect pests. With that realisation, the strain AR37 was released commercially in 2007/08 (Johnson & Caradus 2019). AR37 produces epoxyjanthitrems, which while tremorgenic are not as problematic as lolitrem B (Fletcher et al. 2017). AR37 also protects host ryegrass plants against Argentine stem weevil (adult), pasture mealybug (*Balanococcus poae*), porina (*Wiseana cervinata* or *W. copularis*), African black beetle (*Heteronychus arator*) and root aphid (*Aploneura lentisci*) (Caradus 2023).

The aim here is to describe the ability of a newly discovered *Epichloë* strain, AR128, in protecting ryegrass from insect pests, agronomic performance, animal health and welfare and productivity, food safety, and its transmission to seed and viability in stored seed.

***Epichlo*ë endophyte strain AR128**

Origin

The *Epichloë* strain AR128 was identified using a well-established bioprospecting pipeline deployed at AgResearch (Card et al. 2024). The pipeline aims to identify agriculturally beneficial endophyte strains from germplasm sourced worldwide. AR128 was identified from a 2012 seed collection sourced in Italy.

Known chemistry

As part of the AgResearch bioprospecting pipeline, the chemical profiling of AR128 in the original germplasm showed the production of epoxyjanthitrems, but it was noticed that the proportions of the five epoxyjanthitrem compounds (epoxyjanthitriol, epoxyjanthitrem I, epoxyjanthitrem II, epoxyjanthitrem III, epoxyjanthitrem IV) (Finch et al. 2020) appeared different to that of AR37. Epoxyjanthitrem (EJ) I is known to invoke a sustained tremor response characteristic of lolitrem B in mice but was described as less potent (Finch et al. 2020). It is known that the host genetics can affect the quantity of *Epichloë* endophyte alkaloids produced (Hume et al. 2016). To ensure a reasonably consistent genetic background, AR128 and AR37 were inoculated into the same host germplasm (the perennial ryegrass cultivar Samson) and allowed to mature in the glasshouse. At 7 months post-inoculation, pseudostem tiller sections were harvested from 12 individual plants per endophyte strain and analysed separately using the method described by Hennessy et al. (2016). The analysis (Minitab® 22.2.2, 2025) confirmed that AR128 produced proportionally more EJ II and less EJ I and EJ IV than AR37 (P<0.001) (Figure 1).

Further inoculations into a diploid Italian ryegrass (*Lolium multiflorum* cv. Asset) and a late heading diploid ryegrass (*Lolium* × *hybridum* cv. Platform) provided a wider range of host germplasm in which to evaluate EJ production. This evaluation was undertaken in conjunction with agronomic trials at Ruakura, Waikato and Lincoln, Canterbury with 3 m × 10.6 m plots sampled prior to yield assessments between September 2018 and May 2019. Samples from the replicate plots (n = 4) were analysed as described by Hennessy et al. (2016). As an example, the average concentrations of the individual EJs produced by AR37 and AR128 in Samson in the Ruakura agronomy trial are shown in Figure 2.

Linear regression modelling was used to identify associations between total EJs and endophyte strain using Stata SE version 18 (StataCorp 2023.). The total EJs were log transformed for normality. Sampling date, endophyte strain and an interaction between date and endophyte strain were included in the model. A model was generated for the Ruakura site and the Lincoln site separately. For both Ruakura and Lincoln, endophyte strain (P=0.056, P=0.03, respectively), sampling date (P<0.001, P<0.001, respectively) and the interaction term (P=0.08, P<0.001, respectively) were all significantly associated with the total EJs in a sample, showing that AR128 had lower levels of total EJs than AR37 in the different ryegrass germplasm tested for all sampling dates.

Logistic regression modelling was used to determine the association between endophyte strain and the proportion of the five EJs in a sample using Stata SE version 18. Proportions were transformed using ln(ej/1-ej) to enable modelling of the proportion of EJ per sample. Epoxyjanthitrem variables were retained in the model if the Likelihood ratio test was P<0.05 or odds ratios changes by >5% (indicating confounding). A model was generated for the Ruakura site and the Lincoln site separately. At the Ruakura site EJ I was significantly lower in AR128 (P=0.002) while EJ II was significantly higher in AR128 (P<0.001). For the

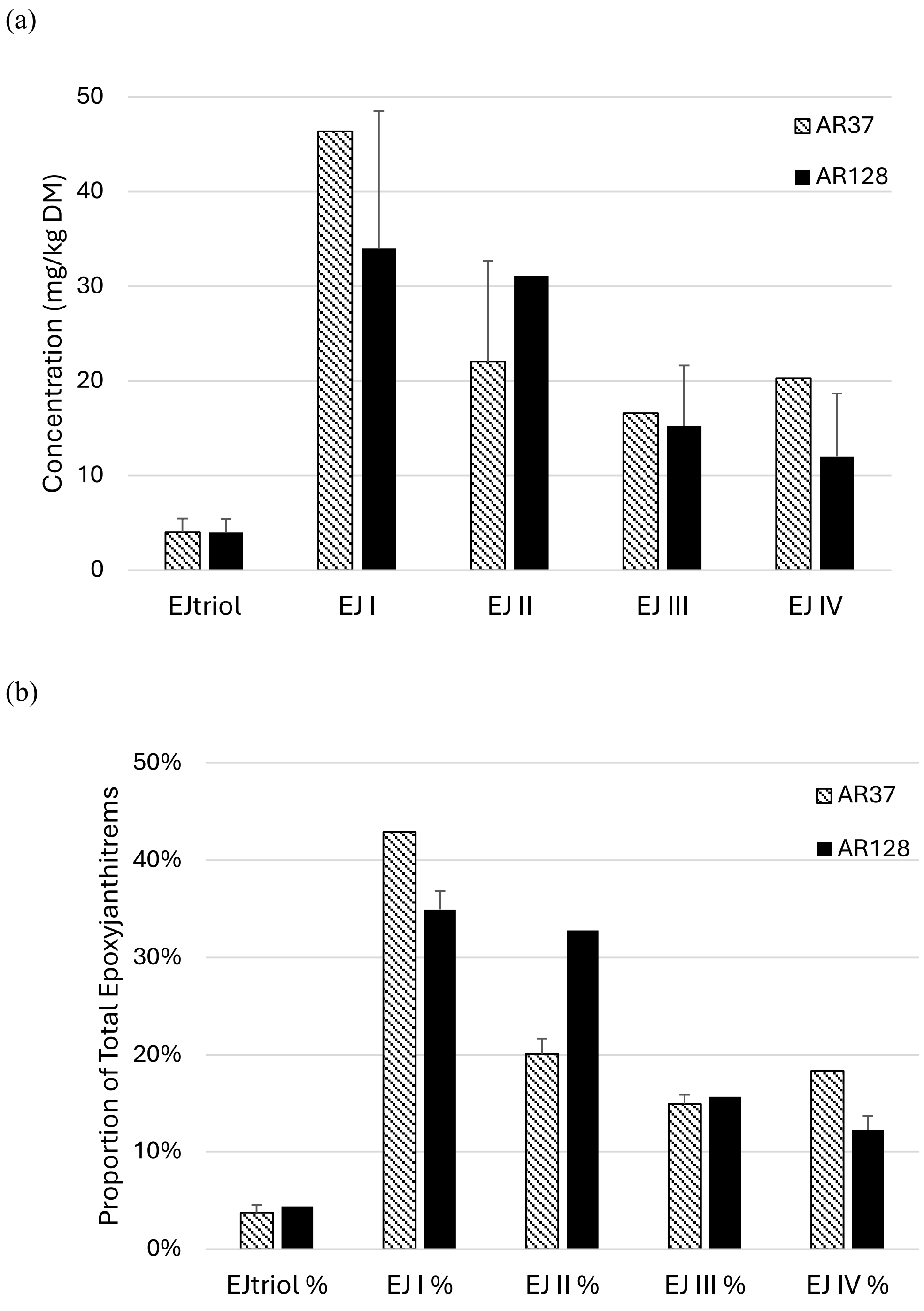

Figure 1 (a) Concentrations (mg/kg DM) and (b) proportions of total epoxyjanthitrems for each individual epoxyjanthitrem compound produced by AR37 and AR128 in perennial ryegrass cultivar Samson grown in a glasshouse and sampled in December 2015. EJtriol = epoxyjanthitriol, EJ I = epoxyjanthitrem I, EJ II = epoxyjanthitrem II, EJ III = epoxyjanthitrem III, EJ IV = epoxyjanthitrem IV. Twelve plants were sampled for each endophyte strain (n = 12). Error bars show the Least Significant Difference between the means within each epoxyjanthitrem compound (95% confidence level).

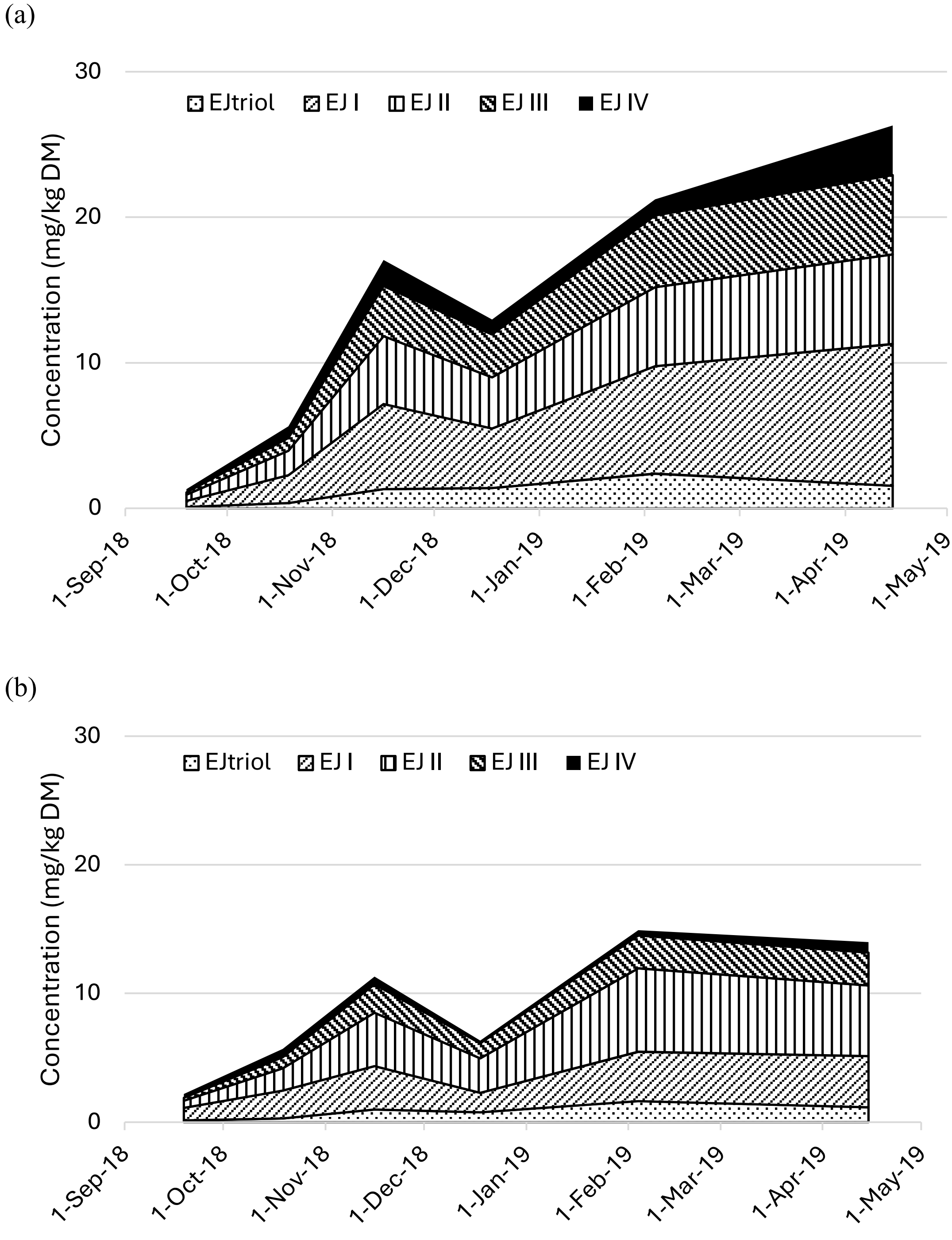

Figure 2 Concentrations (mg/kg DM) of the individual epoxyjanthitrem compounds produced by (a) AR37 and (b) AR128 in perennial ryegrass cultivar Samson in the Ruakura agronomy trials. EJtriol = epoxyjanthitriol, EJ I = epoxyjanthitrem I, EJ II = epoxyjanthitrem II, EJ III = epoxyjanthitrem III, EJ IV = epoxyjanthitrem IV.

Table 1 Average total epoxyjanthitrem concentrations (mg/kg DM) and proportions of each individual epoxyjanthitrem compounds produced by AR37 and AR128 in perennial ryegrass cultivar Samson (measured over 5 years), Asset (measured in 2020), and Platform (measured in 2020), during the late summer and early autumn in grazing trials at Lincoln. EJtriol = epoxyjanthitriol, EJ I = epoxyjanthitrem I, EJ II = epoxyjanthitrem II, EJ III = epoxyjanthitrem III, EJ IV = epoxyjanthitrem IV.

Cultivar × Endophyte	Total EJs	Proportion of each individual epoxyjanthitrem compound				
		EJtriol	EJ I	EJ II	EJ III	EJ IV
Samson AR37	37.16	0.048	0.341	0.243	0.199	0.169
Samson AR128	28.77	0.038	0.274	0.395	0.169	0.123
P value	0.001	0.001	<0.001	<0.001	<0.001	<0.001
Platform AR37	46.75	0.051	0.348	0.230	0.181	0.190
Platform AR128	38.06	0.045	0.284	0.388	0.165	0.118
P value	0.028	0.329	<0.001	<0.001	0.004	<0.001
Asset AR37	45.72	0.050	0.318	0.242	0.201	0.189
Asset AR128	25.08	0.051	0.253	0.422	0.173	0.101
P value	<0.001	0.963	<0.001	<0.001	<0.001	<0.001

Lincoln site there was a significant difference in the proportions of all EJs except epoxyjanthitriol; EJ I (P=0.003), EJ II (P=0.02), EJ III (P=0.003), and EJ IV (P=0.01), with EJ II being the only compound with a higher proportion in AR128 (data is not presented).

During the animal safety and welfare sheep grazing trials the plots were periodically sampled for EJ analysis. One-way ANOVA analyses (Minitab® 22.2.2, 2025) confirmed the previous observations that AR128 produces proportionally more EJ II and less total EJs when compared to AR37 (Table 1).

Genome sequencing

Whole genome sequencing is the process of determining close to the entire DNA nucleotide sequence of an organism's genome with high confidence. Contiguous sequences (or 'contig' meaning a continuous stretch of DNA sequence assembled from overlapping DNA fragments, representing a portion of a larger genome) can be used to determine the degree of genetic difference with other related organisms.

For comparisons of the approximately 34,000,000 base pair genomic sequences of AR37 and AR128 strains, which are evolutionary related members of the same clade, whole genome sequencing and assembly was performed similarly to that described in Miller et al. (2022). To find variant sequences the AR37 genome was used as a reference to which AR128 sequence positions were compared using bioinformatic tools (SAMtools version 1.8 and bcftools version 1.9 (Li 2011)) and AgResearch in-house developed computational scripts.

The types of variants detected were differences in single nucleotide bases (SNPs; single nucleotide polymorphisms) or strings of inserted or deleted nucleotides known collectively as inserted or deleted nucleotides (INDELs). The findings identified approximately 23,000 high-confidence differences between AR37 and AR128, including ~20,000 SNPs and ~3,200 strings of INDELs indicating these strains are genetically different.

Many of the genetic differences between fungal strains can be explained by changes in evolutionary time of nucleotide bases that are not essential for gene function (e.g., SNPs) and the presence/insertion or absence/loss of non-essential accessory single or multiple genes, including large regions encoding for biosynthetic genes producing metabolites that may be bioactive under certain conditions and environments. For comparison, similar genetic alignments of strain AR37 to the distantly related strain AR1 revealed many differences (136,000 SNPs and 23,000 INDELs), whereas alignments to AR40, the most closely related strain in the AgResearch *Epichloë* strain collection to date, found comparatively few changes (719 SNPs and 143 INDELs). Such genetic differences and similarities can be the basis for the observed distinctive and overlapping phenotypic trait sets defining individual strains, including the agriculturally beneficial traits of AR128.

Bioactivity against insect pests

Bioactivity assessment of endophyte strains against major insect pests used, where appropriate, protocols agreed to by the Endophyte Technical Committee of the New Zealand Plant Breeding and Research Association (NZPBRA) (Caradus et al. 2021b). For each of the pot trials, all plants were assessed for the presence of *Epichloë* spp. proteins to confirm presence or absence of the fungus. In all analyses, the significance was assessed by applying a multiple-comparison procedure using the Fisher's protected least significant difference test. Model validation employed graphical diagnostics including residual plots, standardised residuals versus fitted values, and quantile-quantile plots. Where necessary, variables were log or square root transformed to ensure variance stabilisation. For data presentation, all transformed data were back-transformed.

Argentine stem weevil pot trial

Perennial ryegrass plants, cv. Samson either infected with strains AR37 and AR128, or endophyte-free (Nil), were grown from seed at AgResearch, Palmerston North, and sent to AgResearch Ruakura for Argentine stem weevil testing in October 2015. In December, plants were repotted into 12 cm diameter pots with potting mix (Dalton's Ltd, NZ). Sixteen replicate plants per treatment were set up in a randomised block design on a screenhouse bench, under natural temperature and light conditions (n=48 plants). Six adult Argentine stem weevils, collected that day from pasture at Ruakura Research Centre, were caged onto each plant on 23 December 2015.

The number of adult feeding scars on six randomly selected tillers per plant were counted and recorded after 2 weeks. The total number of eggs and the total number of tillers per plant were counted, allowing the calculation of number of eggs per tiller. The cage covers were removed, and larval feeding damage was assessed after a further 4 weeks. Larval damage assessments were undertaken on 25 randomly selected tillers per plant. The number of live and dead tillers checked was in the same proportion of live/dead as on the plant being assessed. Larval damage was scored as: 0 = no damage, 1 = minor damage to the outside tiller, 2 = moderate damage, where larva had penetrated and partially mined the tiller, and 3 = severe damage, where the larva had extensively mined the tiller and/or the meristem had been destroyed. Data were analysed by Generalised ANOVA blocked by replicate with endophyte strain as a treatment factor and number of tillers per plant as a covariate using Genstat 23 (VSN International 2024).

Adult Argentine stem weevil feeding and egg laying did not differ significantly (P>0.05) between the endophyte strains (data not presented). The percentage of tillers damaged by Argentine stem weevil larvae and those with moderate and severe damage was similar for plants infected with AR37 and AR128, with both showing significantly (P<0.001) less damage than Nil (Table 2).

African black beetle pot trial

For the African black beetle trial, perennial ryegrass plants (cv. Samson either infected with strains AR37 and AR128, and Nil) were grown from seed at AgResearch's Ruakura Research Centre in July 2018. Newly germinated seedlings were transferred to polystyrene trays containing potting mix (Daltons Ltd. NZ) and maintained in a glasshouse. In September 2018, 20 replicate plants of each endophyte treatment were transplanted into individual 12 cm diameter pots filled with potting mix. The plants were arranged in a randomised block design within a screenhouse. Prior to trial setup, dead sheath material was removed, and each plant was standardised to 25 tillers.

Adult black beetles were collected over 2 weeks in spring (November 2018) from pitfall traps set in an established pasture on a Waikato dairy farm. Beetles were separated by gender and placed into groups of 20 in containers filled with moist soil and fed every 3 days with fresh organic carrots until the start of the pot trial. One male and one female black beetle were caged onto each plant on 14 November 2018. Plants were watered as required.

Two weeks after caging, black beetle adult feeding was scored for damage to the very base of the tiller: 0 = no damage, 1 = minor damage - surface feeding only, 2 = moderate damage - shredding at the base of tiller but tiller was upright and not wilted, or shredding was higher up the pseudostem, 3 = severe damage - tiller base had been shredded completely, tiller was wilted or dead. Data were analysed by Generalised ANOVA blocked by replicate with endophyte strain as a treatment factor using Genstat 23 (VSN International 2024). Adult African black beetles caused significantly less moderate and severe tiller damage (P=0.0031) in ryegrass plants containing AR128 or AR37 compared to Nil plants (Table 2).

Root aphid pot trial

In October 2015, perennial ryegrass plants of cultivar Samson infected with AR37, AR128 and Nil with 16 replications per treatment were transferred from AgResearch Palmerston North to AgResearch Ruakura (n=48). They were grown in PB ¾ planter bags containing potting mix and maintained in a screenhouse under natural light and temperature conditions. Root aphid assessments were conducted on 10 December 2015 by counting visible root aphid colonies on the outer surface of the root ball between the potting mix and the planter bag.

Data were analysed by Generalised ANOVA blocked by replicate, with endophyte strain as a treatment factor using Genstat 23 (VSN International 2024). The ryegrass plants with either endophyte strain had significantly (P<0.01) fewer aphid colonies than the Nil plants (Table 2).

Porina pot trial

Perennial ryegrass plants (cv. Platform) infected with strains AR37 or AR128 were grown from seed in a glasshouse at Ruakura, Hamilton. Nil plants were generated from an AR37 infected line where seed was held at high humidity and 40°C temperature for 10 days to kill the resident endophyte (Bouton et al. 1993). For each endophyte treatment, five plants of the appropriate endophyte status were transplanted into each of 24 replicate 10 L plastic pots with Yates Thrive potting mix (DuluxGroup Pty Ltd., New Zealand) (n=72). Plants were kept in a screenhouse under natural light and temperature conditions until 17 January 2023, when they were transferred to a controlled temperature room set at 16°C with a 12:12 hour light: dark cycle.

Porina (*Wiseana* sp.) larvae were reared at 15°C from eggs collected at Invermay in November 2022. The larvae were kept in containers with wood chips and fed an artificial carrot-clover based diet (Popay 2001; Hewitt et al. 2025). On 18 January 2023, larvae were sorted by weight and four porina larvae were added to each pot so that larvae within a replicate were of similar weights and mean starting weight increased with increasing replicate number (n=24 replicates). The starting biomass was recorded as mean weight (in milligrams) × four. After 13 weeks the number and percentage of tillers per plant with larval feeding damage was recorded. Surviving larvae were recovered and weighed. The number of surviving larvae recovered per pot varied, so the mean weight gain of the surviving larvae is presented. Non-percentage data were analysed by mixed effect linear regression including treatment, starting porina biomass, and an interaction between treatment and starting biomass. Percentage damage was analysed using a generalised linear model, with a binomial logit link function. The model included treatment, starting porina biomass and an interaction between treatment and biomass. The number of tillers were included as a weighting in the model, and robust error for replicate. Data were analysed using Stata SE version 18.

Percentage tiller damage was lowest in plants infected with AR37 (19%) and AR128 (23%), with no significant difference between them, and highest in Nil plants (26%) (P=0.01). Porina larvae gained the least weight on plants infected with AR37 and AR128 (not significantly different), and the most on Nil (P<0.001). Similarly, porina survival was lower on plants infected with AR37 (31%) and AR128 (42%) than Nil (69%), with no significant difference between the two endophyte treatments (P<0.001, Table 2).

Field trial to test effects on porina

A small plot agronomic field trial near Rakaia, Canterbury (43°41’46.1”S, 171°58’33.8”E), where dead porina were noted on the soil surface, was assessed in 2025 to evaluate the efficacy of several endophyte–perennial ryegrass associations including AR37, AR1, SE and AR128 in several ryegrass cultivars, but did not include cultivar Samson, against porina (*Wiseana* spp.). The trial had been sown on 24 February 2024 and regularly mown for dry matter production. Two sampling methods were used to estimate porina abundance per m² by counting surface tunnels (plastic bag and quadrat). Both methods aimed to provide comparable data, although not all plots could be disturbed, necessitating a dual approach.

The trial was mown on 23 July 2025, and on 25 July 2025 the plastic bag sampling setups were installed in preparation for porina assessment. Within each selected plot (5 × 1.5 m), a diagonal transect was marked, and 10 sampling sites were identified. At each site, a 3–4 cm thick slice of turf was carefully removed using a sharpened 20 cm spade to minimise disturbance to the underlying soil structure. Turf was cut horizontally and kept as intact as possible, creating a clean 20 × 20 cm (0.04 m^2) exposed soil surface. A plastic bag was laid directly over the exposed area, and the turf slice was replaced on top, aligned with its original orientation. The setup was left in place for 5 days to allow porina to re-establish their tunnels beneath the turf. On 30 July, the turf and plastic bag were removed, and visible porina tunnels on the soil surface were counted. Turf slices were then returned to restore the plots. To estimate porina abundance per m^2, tunnel counts from each plot sample area were multiplied by 25. The quadrat method involved placing a 32 cm × 32 cm (0.10 m^2) quadrat along a diagonal transect across the plot. Within each quadrat, porina tunnels on the soil surface were visually identified and counted. To estimate porina abundance per square metre, tunnel counts from each plot sample area were multiplied by 10. The counts from the 10 spade-sized samples within each plot were summed to avoid pseudo-replication. Porina tunnel abundance per m² was analysed using a linear mixed-effects model with ryegrass cultivar and endophyte as fixed effects, and row and column as random effects. Cultivar Three60 infected with AR37 was initially excluded from the statistical design as it was a border plot; however, since row and column effects did not significantly influence the overall analysis, Three60

Table 2 Comparison of endophyte strains AR37 and AR128 against Nil plants in perennial ryegrass cultivar Samson exposed to Argentine stem weevil larvae, African black beetle adults, root aphid and porina in replicated pot trials (porina trial conducted using the ryegrass cultivar Platform). Within rows, means followed by the same letter are not significantly different (P<0.05).

Insect and damage	**Endophyte treatment**			**LSD$_{0.05}$**
	AR37	**AR128**	**Nil**	
Argentine stem weevil larvae				
Damage – all levels (% of tillers)	31 b	21 b	68 a	14
Moderate and severe damage (% of tillers)	15 b	11 b	63 a	15
Adult African black beetle				
Moderate and severe damage (% of tillers) (back transformed log)	13 b	18 b	44 a	NA*
Root aphid				
Number of colonies on outer roots (back transformed log)	0.14 b	0.35 b	11.8 a	NA*
Porina larvae				
Damage (% of tillers)^{A}	19 a	23 a	26 b	NA*
Total porina weight gain (mg)^{B†}	485 a	823 a	1865 b	NA*
Porina survival (%)^{C}	31 a	42 a	69 b	NA*

*NA = not applicable, as a back-transformed LSD cannot be used to compare between back-transformed treatment means

[A] Generalised linear mixed model with a logit link function including treatment, starting porina weight and their interaction was used to analyse the percentage damage

[B] Mixed effects linear mixed model including treatment, starting porina weight and their interaction was used

†of surviving larvae (Total weight gain – larvae start weight)

[C] Chi-square test with a post hoc pairwise comparison; Total percentage

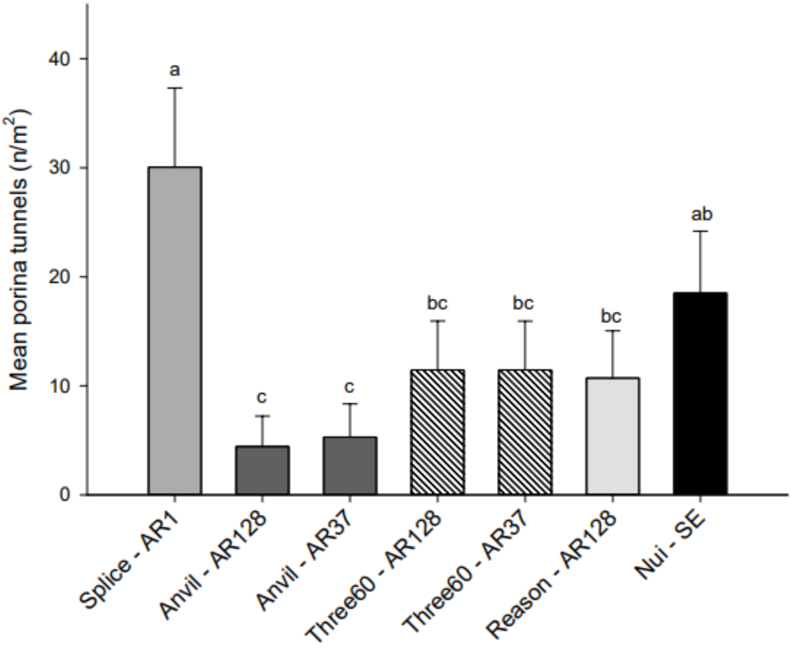

Figure 3 Mean porina tunnel count (n/m²) for ryegrass cultivar by endophyte associations. Endophyte strains include AR1, AR37, AR128, and SE. Error bars represent the standard error of the difference (SED). Means sharing the same letter are not significantly different (P<0.05; Fisher's protected least significant difference test).

Table 3 Endophyte strains AR1, AR37, AR128, SE and Nil in perennial ryegrass cultivars Samson and Platform in a trial at Ōhaupō, Waikato planted in autumn 2023. Comparison were for (1) Argentine stem weevil adults feeding scars per tiller, percentage of tillers per plot with moderate to severe damage (corrected for endophyte-infected tillers only), and percentage of tillers per plot with any larval damage (corrected for endophyte-infected tillers only), (2) African black beetle damage measured as percentage of plants with severe pulling means and standard errors are presented, and (3) plant persistence score in plots taken in May 2025. Within columns, means followed by the same letter are not statistically different (P>0.05).

Endophyte treatment	Argentine stem weevil			African black beetle	Persistence score*
	Mean number adult feeding scars per tiller	% tillers per plot with moderate-severe damage	% tillers per plot with any larval damage	% of plants per plot with severe pulling	
Samson AR1	1.0 ± 0.44 a	10.7 ± 4.6 a	28.6 ± 7.5 a	45.0 ± 10.0 cd	1.5 ± 0.72 c
Samson AR37	1.5 ± 0.47 a	12.1 ± 5.4 a	25.5 ± 7.6 a	20.0 ± 9.6 abc	5.5 ± 0.68 ab
Samson AR128	0.8 ± 0.35 a	3.7 ± 3.9 a	19.5 ± 5.8 a	10.0 ± 9.3 a	6.1 ± 0.68 a
Samson SE	1.2 ± 0.52 a	6.3 ± 5.9 a	22.9 ± 7.7 a	15.0 ± 9.7 ab	5.8 ± 0.70 ab
Samson Nil	3.2 ± 0.48 b	39.3 ± 5.1 b	67.8 ± 8.1 b	52.5 ± 9.8 d	1.1 ± 0.73 c
Platform AR1	0.7 ± 0.44 a	4.9 ± 4.7 a	17.7 ± 7.5 a	37.5 ± 9.7 bcd	2.3 ± 0.69 bc
Platform AR37	0.7 ± 0.42 a	6.9 ± 4.5 a	14.4 ± 7.3 a	22.5 ± 9.4 abc	6.1 ± 0.69 a
Platform AR128	0.6 ± 0.45 a	3.6 ± 4.8 a	25.3 ± 7.8 a	17.5 ± 9.8 ab	5.7 ± 0.72 ab
Test statistics	$X^2_{7,N=25}=45.0$	$X^2_{7,N=25}=75.49$	$X^2_{7,N=25}=58.35$	$X^2_{7,N=32}=33.11$	$X^2_{7,N=32}=107.56$
P value	<0.001	<0.001	<0.001	<0.001	<0.001

* Persistence score has a scale of 1 for a completely dead plot, and 2 = <30% cover to 9 = > 90% cover

AR37 was subsequently included in the statistical model. Statistical analyses were performed in Genstat 23 (VSN International 2024).

Porina numbers were significantly the highest in cultivar Splice AR1 with a mean population of 30 porina per m^2 and lowest in cultivar Anvil AR128 with a mean of 4 porina per m^2 (Figure 3).

Field trial assessment of insect bioactivity - Ōhaupō

An agronomic trial sown in autumn 2023 at Ōhaupō, just southwest of Hamilton, compared insect damage of perennial ryegrass cultivar Samson infected with AR37, AR128, SE or Nil, and cultivar Platform with AR37 and AR128. Potential insect damage was noted in late December 2024-early January 2025, and by mid-January 2025, the effects of the dry summer was apparent with large amounts of dead tillers and plants across the trial. Fifty live, non-reproductive tillers were sampled per plot on 15 January 2025 and assessed for Argentine stem weevil damage. Because of the large number of treatments in the trial, only 3 of the 4 replicate plots were assessed (except for Samson AR128, which had all 4 plots assessed). The number of adult Argentine stem weevil feeding scars, eggs and larvae on each tiller was counted. Larval damage was scored as follows: 0 (no damage), 1 = minor damage to the outside tiller, 2 = moderate damage, where larva had penetrated and partially mined the tiller, and 3 = severe damage, where the larva had extensively mined the tiller and/or the meristem had been destroyed. After assessment, the tiller was assessed for the presence of *Epichloë* spp. proteins. Data were analysed using R version 4.4.2 by linear mixed model fit by REML (R Core Team 2024).

African black beetle larval damage was also assessed in all plots using the ‘pull test’ during the week of 17 February (summer) 2025. This involved randomly throwing a 10 × 10 cm quadrat onto the plot, and the plant within the quadrat “gently” pulled. A plant that did not pull was recorded as a ‘No Pull’. If the plant pulled and there was no evidence of African black beetle larval damage to the roots, it was also recorded as a ‘No Pull’. Where the plant pulled and there was evidence of African black beetle larvae, it was scored as 1 = some root damage, 2 = roots eaten but some roots healthy remain; 3 = roots fully eaten off and plant dead. This was repeated for ten randomly selected plants per plot.

Both Argentine stem weevil and African black beetle damage was similar for plants with AR37, AR128 and SE, and significantly less than for Nil (Table 3). Both Samson and Platform cultivars infected with AR1 showed similar resistance to Argentine stem weevil as cultivars with AR37, AR128 and SE (Table 3). For measures of African black beetle damage percentage of plants per plot with severe pulling was higher for

Samson AR1 than Samson AR128 and SE (Table 3). Persistence after 2 years was scored using a 1 to 9 scale with 1 indicating a dead plot and 2 a plot with <30% ryegrass cover to 9 indicating >90% ryegrass cover. The percentage score relates to the amount of the plot covered by the original population sown; this was measured immediately post-grazing to ensure there were no canopy effects on scoring. While overall persistence for a 2-year-old sward was poor due to severe drought in 2024/25 (Metservice 2025), both AR37 and AR128 conferred the greatest persistence in both Samson and Platform cultivars along with SE in Samson, compared with Nil and AR1 (Table 3).

Animal safety and welfare assessment with sheep

AR128 has undergone animal safety testing with 6-month-old weaned Romney ewe lambs over five February/March periods (2019–2021, 2024 and 2025) and during three successive seasons of 2021 calendar year (May/June, September/October, November/December). All manipulations on sheep were approved by the AgResearch Animal Ethics Committees.

New pastures were sown in the spring before testing each year. Irrigation was available to ensure good grass establishment and growth across the trial area. The 2021 trial compared treatments over three seasons through utilising the regrowth of pastures initially grazed in the summer/autumn 2021. In addition to AR128, SE was the positive control as it is known to have a high degree of mammalian toxicity and Nil (no mammalian toxicity) was the negative control. Inclusion of AR37 and AR1 treatments provided comparison to commercially available endophytes with varying levels of mammalian toxicity (SE>AR37>AR1>Nil). In 2020, the Nil treatment was omitted due to a lack of land availability. During this year, AR1 represented the negative control. In 2025, no AR1 comparative control was included for the same reason. Endophyte treatments were all hosted by a common diploid perennial ryegrass cultivar (Samson).

The testing largely followed the sheep testing model of Fletcher et al. (2017). This meets the requirements of the Endophyte Technical Committee, NZPBRA (Caradus et al. 2021b), a committee which approves grass × endophyte association ratings for animal health and safety. These requirements stipulate thresholds for endophyte infection of pastures (>85% of tillers) and differences between measured variables of positive and negative control treatments to qualify as a valid test. Grass availability per sheep was standardised across plots with the addition of extra sheep as required. The animal variables measured during this testing included sheep live weight gain, ryegrass staggers (scored using the Keogh scale – Keogh 1973), susceptibility to heat stress, and dag accumulation. Sheep were also regularly observed for general health and well-being. In addition to endophyte infection measurements of pasture treatments, endophyte alkaloid concentrations of the pastures grazed by sheep were measured. Statistical analysis of ryegrass staggers data used a linear mixed effects model conducted using R version 4.5.0, along with 'predictmeans' package. Live weight gain, respiration rate and rectal temperature data from 2019 to 2021 underwent analysis of variance using Genstat 18th edition. Live weight gains, respiration rates and rectal temperatures in 2024 and 2025 were analysed via mixed effects model, all using R version 4.3.3, along with 'predictmeans' and 'lme4' packages.

The February/March grazing periods generated considerable mammalian toxicity towards livestock on the SE treatment each year (Tables 4-6). In 2021, no endophyte effect was detected on any treatments in either the May/June or September/October grazing periods. For this reason, the data is not presented. This was not unexpected as alkaloid concentrations measured in the herbage were far lower than those measured in February/March (data not shown). Alkaloid concentrations in herbage increased in the November/December period and an endophyte effect of the treatments was again measured. Across five February/March periods, ryegrass staggers symptoms were similar (P≥0.063) for AR128 and AR37 each year and when averaged over all years (Table 4). AR128, like AR37, was consistently more (P≤0.018) toxic to livestock than AR1 and less (P≤0.021) toxic than SE except for 2020 when SE, AR128 and AR37 were not significantly different (P≥0.089).

During February/March trials, live weight gain of sheep grazing AR128 was similar to that of AR37 (Table 5). At the mid-trial point for four out of five trials the live weight gain of sheep on AR128 were greater (P≤0.044) than on SE but AR37 and AR128 were never different (P≥0.294) from each other. Live weight gain of sheep grazing AR128 was less (P≤0.043) than both Nil and AR1 during 2019 and 2020, but not different (P≥0.053) in other years. The final results were more impacted by the requirement to remove sheep due to their ryegrass staggers symptoms exceeding a predefined threshold. AR128 live weight gain was greater (P=0.049) than SE in 2019 and less (P=0.039) than AR1 in 2021 but otherwise not different (P≥0.111) to other treatments.

AR128 was tested during four heat stress assessments which detected statistical differences in rectal temperature and respiration rates between sheep grazing SE, which is known to exacerbate heat stress,

Table 4 Predicted mean ryegrass staggers (±SEM) using the 1 to 5 scale of Keogh (1973) following five weeks of sheep grazing AR1, AR37, AR128 and SE) in perennial ryegrass cultivar Samson over five February/March periods and the average over five years. Within columns, means followed by the same letter are not statistically different (P>0.05).

Endophyte treatment	**Year and months (ryegrass staggers score)**					
	2019 Feb/Mar	**2020 Feb/Mar**	**2021 Feb/Mar**	**2024 Feb/Mar**	**2025 Feb/Mar**	**Average over 5 years**
AR1	0.3 ± 0.3 c	2.2 ± 0.3 b	0.3 ± 0.3 c	0.9 ± 0.3 c	NR*	0.9 ± 0.1 c
AR37	2.6 ± 0.3 b	4.0 ± 0.3 a	1.5± 0.3 b	2.3 ± 0.3 b	1.4 ± 0.3 b	2.4 ± 0.1 b
AR128	2.5 ± 0.3 b	3.3 ± 0.3 a	1.4 ± 0.3 b	2.8 ± 0.3 b	2.1 ± 0.2 b	2.4 ± 0.1 b
SE	3.6 ± 0.3 a	4.0 ± 0.3 a	3.6 ± 0.3 a	4.0 ± 0.3 a	3.4 ± 0.2 a	3.7 ± 0.1 a

*NR-Treatment was not represented during this year.

Table 5 Mean live weight gain (LWG) (±SEM) to mid-trial and end of trial of sheep grazing Nil, or plants infected with AR1, AR37, AR128 and SE treatments in perennial ryegrass cultivar Samson over five February/March periods. In each year, within columns, means followed by the same letter are not statistically different (P>0.05).

Treatment and year	**Mean LWG from beginning to mid-trial (g/day)**	**Mean LWG from beginning to end of trial (g/day)**
2019		
Nil	45 ± 19.7 ab	24 ± 9.3 ab
AR1	73 ± 23.7 a	40 ± 10.6 a
AR37	-2 ± 31.3 bc	18 ± 18.4 ab
AR128	-10 ± 7.1 c	40 ± 5.8 a
SE	-42 ± 18.0 c	-14 ± NA† b
2020		
AR1	281 ± 34.2 a	179 ± 13.3 a
AR37	76 ± 33.7 b	NM*
AR128	122 ± 81.6 b	186 ± 30.6 a
SE	-63 ± 18.8 c	NM
2021		
Nil	241 ± 34.2 a	141 ± 17.7 ab
AR1	286 ± 34.5 a	154 ± 17.8 a
AR37	273± 36.5 a	83 ± 22.4 b
AR128	234 ± 30.6 a	101 ± 15.8 b
SE	102 ± 34.4 b	NM
2024		
Nil	295 ± 27.0 a	166 ± 8.5 a
AR1	245 ± 16.6 a	156 ± 10.8 a
AR37	268 ± 17.3 a	157 ± 11.0 a
AR128	240 ± 25.6 a	126 ± 44.0 a
SE	135 ± 26.0 a	NM
2025		
Nil	132 ± 28.9 a	80 ± 16.3 a
AR37	120 ± 28.9 a	63 ± 16.3 a
AR128	111 ± 28.9 a	48 ± 16.3 a
SE	60 ± 28.9 b	NM

†No standard error of the mean was available for SE in 2019 with only one replicate represented because all other sheep had been removed having reached ryegrass staggers score of 4.

*NM – no measurements taken because sheep had been removed from this treatment having reached ryegrass stagger rating of 4.

Table 6 Mean rectal temperature and respiration rate (±SEM) of sheep grazing Nil endophyte plants or plants infected with AR1, AR37, AR128 and SE treatments in perennial ryegrass cultivar Samson at four assessments. At each assessment, within columns, means followed by the same letter are not statistically different (P>0.05).

Treatment and year	Mean rectal temperature (°C)	Mean respiration rate (b/min)
2021 February		
Nil	40.4 ± 0.06 b	172 ± 2.2 c
AR1	40.4 ± 0.07 b	174 ± 2.6 c
AR37	40.5 ± 0.08 b	184 ± 1.8 b
AR128	40.5 ± 0.06 b	182 ± 2.2 b
SE	40.7 ± 0.11 a	194 ± 2.0 a
2021 December		
Nil	40.4 ± 0.07 c	170 ± 2.0 c
AR1	40.4 ± 0.07 bc	172 ± 2.0 c
AR37	40.5 ± 0.07 abc	182 ± 2.0 b
AR128	40.6 ± 0.06 ab	181 ± 1.8 b
SE	40.7 ± 0.07 a	190 ± 2.0 a
2024 February		
Nil	40.0 ± 0.08 b	220 ± 8.8 b
AR1	40.2 ± 0.07 b	223 ± 8.7 b
AR37	40.1 ± 0.07 b	233 ± 8.7 b
AR128	40.2 ± 0.07 ab	239 ± 8.7 b
SE	40.5 ± 0.08 a	277 ± 9.0 a
2025 February		
Nil	40.0 ± 0.18 b	190 ± 9.0 b
AR37	39.9 ± 0.18 b	207 ± 8.9 ab
AR128	39.9 ± 0.18 b	215 ± 9.0 a
SE	40.3 ± 0.18 a	224 ± 9.1 a

and Nil. These resulted in all measurements being similar ($P \geq 0.418$) for AR128 and AR37 (Table 6). In December 2021, rectal temperature of sheep grazing AR128 was greater ($P=0.036$) than for sheep grazing Nil and not different ($P=0.123$) to SE. In 2024, the rectal temperature of AR128 sheep was intermediate to SE and Nil and not different ($P \geq 0.059$) to either. In 2025, respiration rate of sheep grazing AR128 was greater ($P=0.033$) than Nil and not different ($P=0.949$) than SE. Other AR128 measurements of respiration and rectal temperature were less ($P \leq 0.027$) than SE (Table 6).

For the animal testing trials the ratio of EJ compounds was consistently different between AR128 and AR37 ($P<0.002$). The EJ I concentration was higher in AR37 than AR128 herbage ($P<0.001$) whereas the EJ II concentration was higher in AR128 than AR37 herbage ($P<0.001$) (Table 1). The concentrations of total EJs were affected by year as would be expected under different environmental conditions. However, AR128 and AR37 did not respond consistently with differences between them in EJ production observed under different environmental conditions.

Animal safety and welfare assessment with mice

Mice were used as a cost effective and rapid screening method for determining the likelihood of endophyte-grass associations to induce ryegrass staggers in the field. Ground seed (AR37 or AR128) was extracted with 2:1 dichloromethane-methanol and preliminary purification achieved using liquid-liquid partitioning and flash column chromatography to remove toxic fatty acid compounds. Extracts were administered to mice intraperitoneally as dimethylsulphoxide solutions (50 µL) and tremorgenicity measured using a visual rating scale as previously described (Gallagher & Hawkes 1986; Munday-Finch et al. 1997). All animal manipulations were approved by the AgResearch Animal Ethics Committee.

Analysis of the AR128 and AR37 extracts showed the concentrations of EJs to be very similar (73.2 and 74.5 mg/kg for AR128 and AR37 seed, respectively). These AR128 and AR37 extracts induced comparable tremor responses in mice, with the time course of action

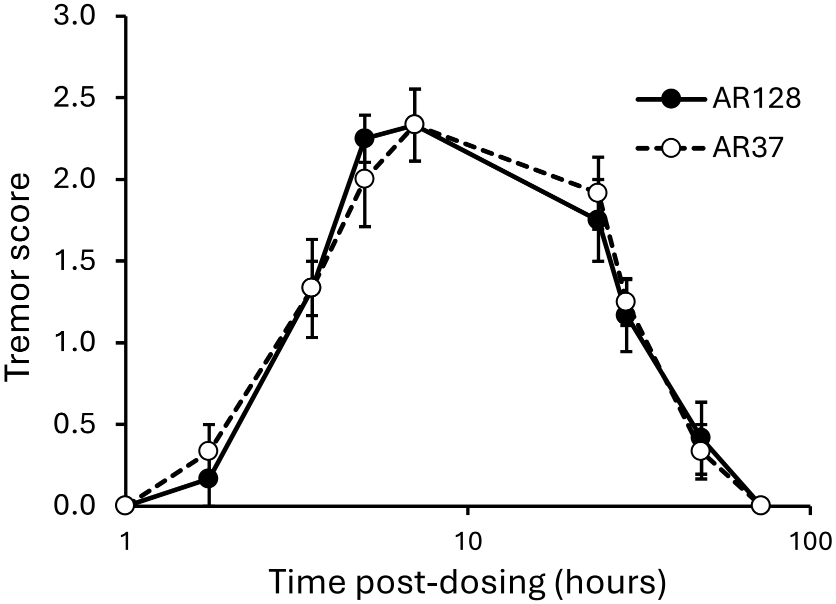

Figure 4 Mouse tremorgenicity screen of AR128 and AR37 showing tremor score vs time post-dosing (hours). Error bars represent standard errors of the means.

consistent with those being caused by EJs (Figure 4). These results suggest that there are no unknown tremorgens present in AR128 which are not present in AR37.

Food safety assay

Food safety studies are conducted on new endophytes of commercial interest with the knowledge that a new endophyte will undoubtedly express new compounds which have the potential to go into the food chain to be consumed by humans. A food safety study using mice determines whether any compound expressed by the endophytes poses a risk to human health.

Sub-acute toxicity was assessed by feeding groups of 3-week-old mice (5 male and 5 female) a diet containing 30% AR128 seed for 21 days using a feeding protocol designed to replicate human feeding behaviour (Finch et al. 2022). An analogous group of mice was fed a diet containing 30% endophyte-free seed as a control. Diet was prepared using established methods (referenced above) and the trial protocol were consistent with that used previously (Finch et al. 2021). Food consumption and bodyweight was measured daily and motor coordination, grip strength, blood pressure and heart rate were assessed weekly. At the end of the trial period mice were euthanised and a blood sample taken for analysis of plasma enzymes and haematological parameters. Major organs were weighed and samples taken for histopathological examination. All animal manipulations were approved by the AgResearch Animal Ethics Committee.

There was no difference between the mice fed a diet containing endophyte-free seed and those fed a diet of seed containing AR128. There were no toxicologically significant differences in the heart rates, blood pressures, haematology or organ weights of mice fed either endophyte-free seed or seed infected with AR128. Endophyte treatment had no effect on body weight, motor coordination or grip strength of the mice.

Transmission, viability and storage

Seed from three years of seed multiplication of ryegrass cultivars Samson and Platform with AR128 was assessed for viable endophyte frequencies either immediately after seed harvest, drying and dressing, or if delayed the seed was stored at 0–3°C and 30% relative humidity. This demonstrated that levels of viable endophyte of AR128 in successive seed multiplication events had transmission at a level acceptable for industry with very little decline in endophyte viability in the two ryegrass cultivars (Table 7).

Separately, vertical endophyte transmission frequency was assessed by checking 24 seedlings from each family of 10 different ryegrass cultivars using an RNA high-resolution melt (HRM) method carried out by Slipstream Automation, Palmerston North, New Zealand (Gagic et al. 2018). Raw vertical transmission frequency for AR37 was 95% and for AR128 was

Table 7 Viable endophyte infection frequencies (%) of seed multiplications of AR128 in ryegrass cultivars Samson and Platform using established methods for endophyte detection (Simpson et al. 2012). Each seed generation used seed directly from the previous increase with no reselection between seed generations to improve the endophyte infection frequency.

Year	Cultivar	
	Samson	Platform
1	99	98
2	99	98
3	96	No harvest taken

96%. 'Raw' transmission is an unweighted (i.e. no adjustment for the proportional contribution of each family to the final bulk) mean transmission frequency of all the families that were harvested.

A seed storage experiment was initiated in April 2021 near Lincoln, New Zealand. This experiment utilised seeds of Samson perennial ryegrass containing AR37 (three seed lots) and AR128 (two seed lots). Small samples of seed from each seed lot (~2.5 g/seed lot) were placed in paper packets in a container that ensured all seed packets were equally exposed to ambient air temperature and humidity for a 23-month period (April 2021 and March 2023) - temperature mean 12.6°C (daily average minimum 7.8°C and maximum 17.2°C) and mean relative humidity of 79.7% (daily average minimum 58.8% and maximum 94.0%). Sub-samples were removed at approximately 3-month intervals and assessed for their viable endophyte infection frequencies by growing 100 seedlings using the established method of Simpson et al. (2012). To identify differences between endophyte strains, 83.4% confidence intervals were generated for endophyte viability (proportion) for each endophyte and date, using a z score of 0.166 (Cave 2022). These data were presented visually, with lines between each timepoint smoothed using Lowess smoothing. Differences between AR128 and AR37 endophyte viability frequencies were modelled using a generalised linear model with a binomial function and robust variance including endophyte strain and date. AR128 stored better than AR37 (P<0.001) for every examination timepoint in the 23-month period (P<0.001) except 2 December 2021 (P=0.07) (Figure 5). Statistical analyses were conducted using Stata SE version 18.

Agronomy

From autumn 2018 at the AgResearch Ruakura Research Farm, Waikato, perennial ryegrass cultivars and experimental cultivars with selected and SEs were evaluated in 1.5 m × 6 m plots. The cultivars and experimental synthetic cultivars were randomised in a row-column design with three replicates. Plots were sown at 20 kg/ha and grazed by sheep when pre-grazing pasture mass was approximately 2400–3000 kg DM/ha. Ground cover scores (1 – 9; representing <20% to >90%

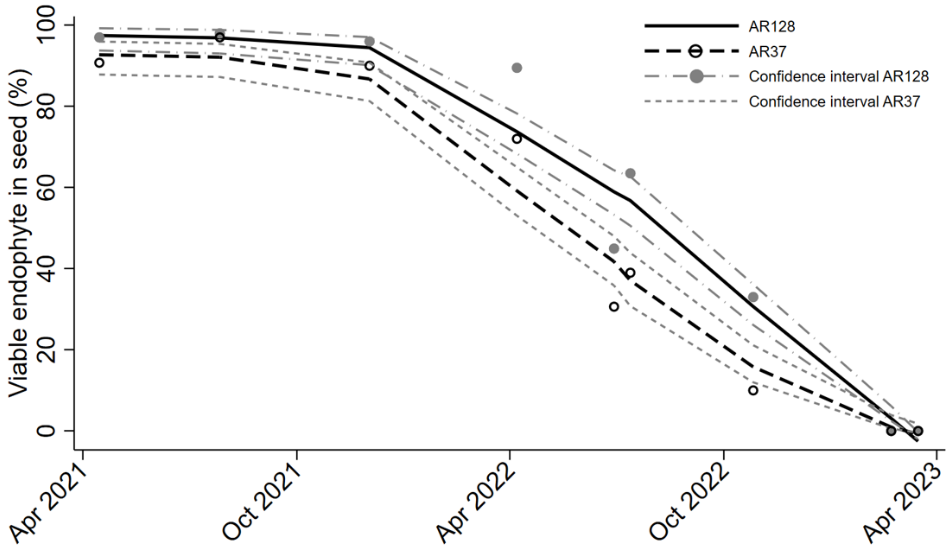

Figure 5 The smoothed percentage and 83.4% confidence interval for seeds with viable endophyte frequencies for AR128 and AR37 stored under ambient conditions between April 2021 and March 2023. Fitted curves used Lowess smoothing and circles indicate actual values.

Table 8 Comparison of endophyte treatments in perennial ryegrass cultivar Samson at Ruakura, Hamilton after drought in Year 2 for persistence (ground cover score) and percentage of plants damaged per plot by African black beetle larvae on 2 May 2020, and in Year 3 ground cover score after drought on 16 April 2021 (Year 3) and winter recovery on 3 June 2021 (Year 3). Means that have the same letter (based on Duncans P>0.05) are not significantly different.

Endophyte treatment	Post-drought recovery – year 2			Post-drought recovery - year 3	Winter r ecovery - year 3
	Ground cover score*	Plants with black beetle damage (%)	Plants with moderate/ severe pulling (%)	Ground cover score*	Ground cover score*
AR1	4.3 b	44 a	33 a	1.4 cd	1.5 d
AR37	7.9 a	33 a	33 a	4.4 b	5.7 ab
AR5	8.2 a	22 a	11 a	5.4 b	7.2 a
AR127	7.5 a	50 a	44 a	2.7 c	3.4 c
AR128	8.5 a	17 a	17 a	4.6 b	5.5 b
SE	8.2 a	28 a	22 a	4.2 bc	6.2 a
Nil	1.0 c	89 b	89 b	1.1 d	0.9 d
P value	<0.001	0.016	0.030	<0.001	<0.001
$\text{LSD}_{0.05}$	1.5	36.4	42.8	1.5	1.8

* Ground cover score has a linear scale of 1 = <20% to 9 = 90 to 100%

vegetative ground cover) were noted in May 2020, April 2021 and June 2021. In addition, plant damage caused by root feeding of African black beetle larvae was assessed in May 2020 by measuring six samples per plot for the absence or presence of "pulling".

An optimal REML spatial model was fitted to each trial measurement using Genstat 24 (VSN International 2024). The model with the minimal Bayesian Information Coefficient (Gilmour et al. 1997) was selected as the optimal model. Comparisons were made of endophyte strains AR128, AR127 (another EJ producing endophyte), AR1, AR5 (an ergovaline producing endophyte), AR37, SE, and Nil all in perennial ryegrass cultivar Samson (Table 8 and Figure 6).

Nil plots had significantly less (P<0.001) ground cover by May 2020 (autumn Year 2) than all endophyte infected treatments. By autumn Year 3, both Nil and AR1 plots were indistinguishable from one another and had a lower ground cover score (P<0.001) than all hosts with both EJ producing and ergovaline producing endophytes (P<0.001), except AR127. AR127 had lower ground cover scores than AR128, AR37, AR5 and SE at the end of Year 3 (P<0.001).

All endophyte treatments had a lower African black beetle damage score than the Nil treatment (Table 8). The strong correlation in Year 2 between ryegrass persistence (measured as ground cover score) and percentage of plants damaged by African black beetle would suggest a possible causal link (Figure 7). In the third year after both insect attack and summer moisture stress, the best recovery was seen in plots with AR5, AR37, AR128 and SE with about 40 to 50% ground cover which was maintained 2 months later when scored for winter recovery (Table 8).

Discussion

Over the past 40 years, research on asexual *Epichloë* spp. within temperate ryegrasses in New Zealand has led to the discovery and characterisation of selected *Epichlo*ë strains. These fungi provide protection to the ryegrass plant from insect pests and simultaneously reduce animal health and welfare concerns resulting from mammalian toxic chemistry expressed by the endophyte (Caradus 2023). Selected *Epichloë* strains can also improve productivity through increased meat and milk production compared to the more toxic SE (Bluett et al. 2005; Fletcher 2005). Now almost all proprietary ryegrass cultivars sold and used in New Zealand pastoral agriculture contain one of these selected endophytic strains (Caradus et al. 2021b; Hewitt et al. 2021).

The advent of AR37 in 2007/08 provided ryegrass with excellent protection against most of the insect pests that negatively impact grass persistence in New Zealand (Hume et al. 2007). AR37 was characterised by not expressing any of the then known mammalian toxins but did express EJs (Tapper & Lane 2004).

The new selected strain AR128 also expresses EJs but in slightly different proportions to that observed for AR37. AR128 protects the ryegrass plant against the same insect pests as AR37, namely Argentine

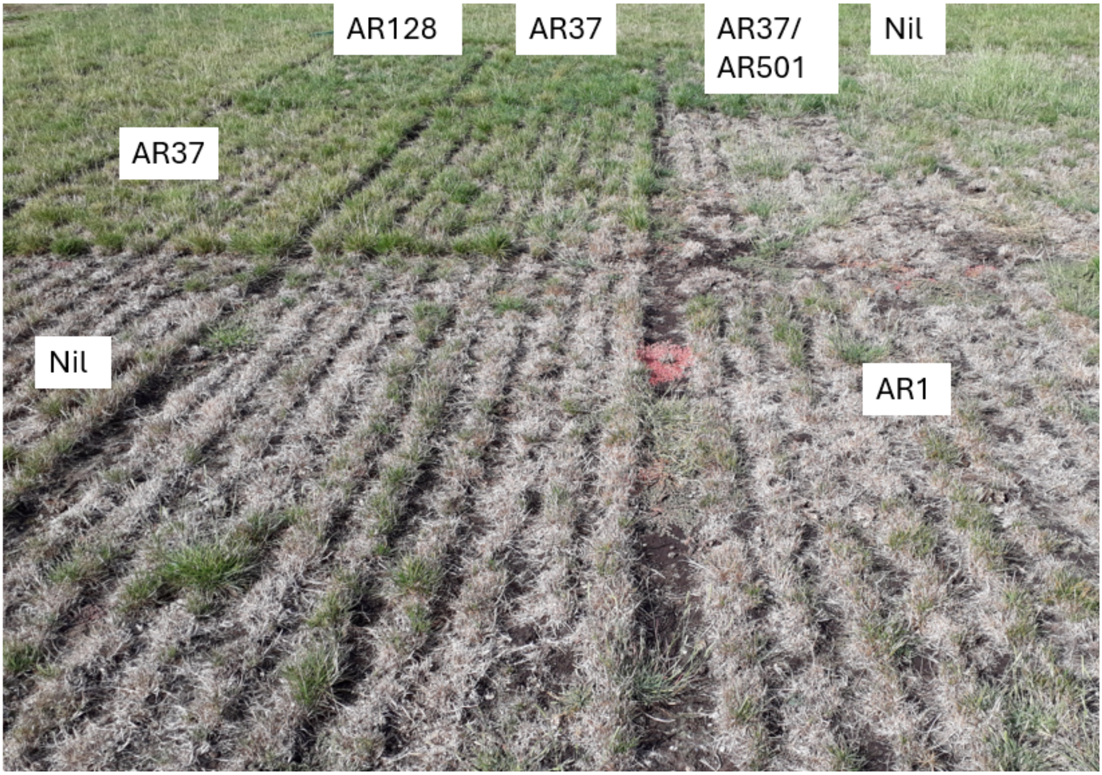

Figure 6 Photo taken on 10 March 2020 at Ruakura after the trial had been through its second summer.

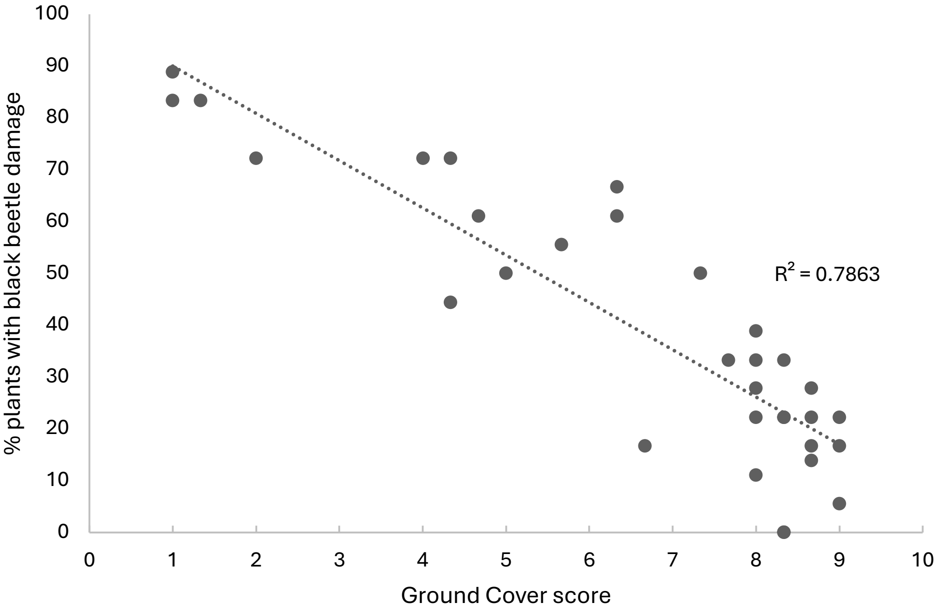

Figure 7 Relationship between percentage of plants with African black beetle larval damage and persistence measured as ground cover score for year 3 of the 2018-sown Ruakura agronomy trial. This figure includes the 7 endophyte × cultivar treatments listed in Table 8 plus another 26 combinations included in the trial.

stem weevil, African black beetle, root aphid and porina. AR128 also exhibited similar animal safety effects as AR37. However, it transmits through seed production cycles at a greater frequency than AR37 and stores for a longer duration in seed at ambient conditions, ensuring that the end-user farmer obtains a high quality and effective endophyte product. This is important, as the New Zealand proprietary pasture and forage seed industry have adopted a position that for endophytic ryegrass and fescue seed to be sold there is an expectation that at least 70% of seed will be infected with viable endophyte at the time of sale to the retailer. The significance of this is that agronomic performance can be positively correlated with the frequency of endophyte infection (Valentine et al. 1993; Popay et al. 1999; Hume & Sewell 2014).

Practical implications

AR128, a new *Epichloë* product for ryegrass, provides good protection (like AR37) against major insect pests even through drought events without exacerbating animal health and welfare concerns. It both transmits and stores well in seed even at ambient temperatures and humidity, ensuring a high quality and effective endophyte technology for pastoral farmers.

ACKNOWLEDGEMENTS

To Jaspreet Singh and Wei Zhang for preparing AR128 DNA and RNA for the genomics study; to Leo (Xinqi) Liu, Yulia Morozova, Nikki Webb, Jan Sprosen and Jacob Shrubsall for contributions to the sample analysis for the chemistry results; and to Alexia Becroft for agronomic support.

Disclosure statement

John Caradus is employed by Grasslanz Technology Limited, a company that is a part-owner of the intellectual property associated with the *Epichloë* strain AR3002 and *Epichloë* strains marketed under the brands AR1®, AR37®, AR128®, Endo5®, MaxQ®, MaxQII®, MaxP®, MaxR®, Avanex®, Happe® and Protek®. Richard George, Brian Maw, and Michael Norriss are employed by PGG Wrightson Seeds Limited which is the part-owner of and also produces and markets grass endophytic cultivars that may contain AR1®, AR37®, AR128®, Endo5®, MaxQ®, MaxQII®, MaxP®, MaxR®, Avanex®, Happe® and Protek®. David Hume, Alison Popay and Wade Mace are inventors on the AR128® patent and David Hume is an inventor on the AR3002 patent.

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