

AR153 – a new *Epichloë* fungal endophyte for ryegrass

Katrin G. HEWITT¹, John R. CARADUS², David E. HUME¹, Joanne G. JENSEN¹, Alison J. POPAY¹, Anouck C. M. DE BONTH¹, Russell G. CROY³, Justine FERGUSON⁶, Natasha T. FORESTER¹, Richard M. GEORGE⁴, Sarah M. ROSANOWSKI¹, Paul H. MACLEAN¹, Dongwen LUO¹, Wade J. MACE¹, Marty J. FAVILLE¹, Michael G. NORRIS⁴, Brian MAW⁵, Sarah C. FINCH¹ and Alan STEWART⁴

¹Bioeconomy Science Institute, Hamilton, New Zealand

²Grasslanz Technology Ltd., Palmerston North, New Zealand

³Bioeconomy Science Institute, Christchurch, New Zealand

⁴PGG Wrightson Seeds Ltd., Lincoln, New Zealand

⁵PGG Wrightson Seeds Ltd., Ruakura Research Centre, Hamilton, New Zealand

Corresponding author: kati.hewitt@agresearch.co.nz

Abstract

Perennial ryegrass (*Lolium perenne* L.) persistence in many parts of New Zealand is reliant on its association with the mutualistic *Epichloë* fungal endophyte. This endophyte protects ryegrass from various insect pests and can improve tolerance to some abiotic stresses such as drought. Over the last 25 years several selected endophyte strains have been commercialised. These strains have improved ryegrass persistence and minimised the negative impacts on livestock of ryegrass staggers, heat stress and productivity losses associated with the standard endophyte strain. A new endophyte strain is becoming available – AR153, as a replacement for AR1. This *Epichloë* strain from Italy has a similar known chemical profile to AR1, through expression of peramine, but it also produces chanoclavine. AR153 protects ryegrass against the same insect pests as AR1. It exhibits improved or similar animal safety, high transmission to seed, improved storage in seed and similar yield and persistence compared with AR1. No food safety concerns have been identified. These traits indicate that AR153 is an effective replacement for AR1, supporting greater ryegrass persistence than endophyte-free pastures while reducing the incidence of ryegrass staggers.

Keywords: alkaloids, animal welfare, insect pests, storage, transmission

Introduction

Perennial ryegrass (*Lolium perenne* L.) is the cornerstone of temperate pastoral systems in New Zealand, where pasture persistence is threatened by the combined pressures of insect herbivory, episodic drought and intensive grazing. Symbiosis with asexual *Epichloë* endophytes is a key mechanism underpinning ryegrass resilience across New Zealand (Johnson et al. 2013; Caradus 2023; Hewitt et al. 2025a). These endophytes are obligate mutualists, occupying intercellular spaces of above ground tissues and passing vertically to the next generation of plants through seed (Christensen et

al. 2002; Zhang et al. 2022). Their agronomic value derives largely from the production of secondary metabolites with defensive functions that act as feeding deterrents or toxins against invertebrates, with some compounds also affecting grazing livestock (Caradus & Johnson 2020). *Epichloë* endophytes can produce four well-defined alkaloid classes with distinct biosynthetic pathways and bioactivities: 1-aminopyrrolizidines (e.g., *N*-formyllooline), pyrrolopyrazines (e.g., peramine), ergot alkaloids (e.g., ergovaline) and indole-diterpenes (e.g., lolitrem B) (Scharldt et al. 2012; Realini et al. 2024).

Historically, the widespread “standard”, or common toxic, endophyte (SE) exemplified a trade-off between agronomic and animal performance. While providing strong insect resistance, SE produces lolitrem B and ergovaline, compounds associated with ryegrass staggers and exacerbation of heat stress in livestock, respectively, leading to reductions in milk and meat production (Fletcher et al. 1999; Di Menna et al. 2012; Card et al. 2024). These findings started the search for animal-safe selected endophyte alternatives. Strategic selection and deployment of endophyte strains therefore aim to balance two imperatives: robust and durable protection against key pasture pests, and minimisation of adverse outcomes for animal health, welfare and productivity (Caradus et al. 2021).

A pivotal outcome was the AR1 selected endophyte strain, released in 2001 (Easton et al. 2001). AR1 lacks lolitrem B and ergovaline yet retains peramine and confers effective deterrence of Argentine stem weevil ASW (*Listronotus bonariensis*) and pasture mealybug (*Balanococcus poae*) (Pennell et al. 2005; Ruppert et al. 2017). AR1 was rapidly adopted into New Zealand farming systems, and by 2008 approximately 70% of all proprietary perennial ryegrass seed sold incorporated this endophyte strain (Caradus et al. 2013).

No currently commercialised perennial ryegrass endophyte provides both complete and consistent suppression across the full pasture pest complex and complete mitigation of negative animal health

and welfare outcomes under all environmental conditions (Hewitt et al. 2025a), in addition to good transmission and viability in seed during storage. Residual vulnerabilities persist, and performance can vary with climate, soil fertility, grazing management and interannual stress patterns (Caradus 2023; Card et al. 2024). Consequently, there is continued demand for next-generation strains that: (i) expand or refine the spectrum of invertebrate deterrence, (ii) sustain agronomic performance under environmentally challenging conditions and grazing pressure, (iii) eliminate/minimise livestock toxicoses and (iv) meet operational requirements for food safety, animal welfare and reliable delivery of the technology to the end user (seed transmission and storage).

To address these challenges, AR153, an *Epichloë* strain, was developed as a replacement for AR1, with the aim of maintaining the combination of animal safety, insect protection and pasture performance associated with AR1. Unlike strains such as AR37, which provide broader insect control, but may have greater impacts on animal health and welfare, AR153 was developed to prioritise animal safety while maintaining effective insect protection. This manuscript describes the performance of AR153 across five aspects relevant to on-farm performance.

- Insect pest protection: spectrum, magnitude and consistency of deterrence/suppression.
- Animal health and welfare: absence of problematic vertebrate-active alkaloids.
- Food safety: conformance with relevant standards and residue expectations.
- Seed transmission and storage viability: vertical transmission fidelity and maintenance of endophyte viability during storage.
- Agronomic robustness: persistence, growth and sward stability under grazing and abiotic stresses.

The aim here is to describe the characteristics of AR153, including its effects on insect pest protection, pasture productivity, animal health and welfare, food safety, transmission and viability in seed, and to position AR153 within the current endophyte strain landscape.

Origin

The *Epichloë festucae* var. *lolii* endophyte strain AR153 was identified through AgResearch's bioprospecting pipeline for endophyte discovery (Card et al. 2024). This programme surveys globally collected germplasm to identify strains with agronomic potential with a focus on animal health, insect deterrence and pasture productivity. AR153 was identified from a 2012 perennial ryegrass seed collection sourced in Italy.

Known *Epichloë* endophyte chemistry

The chemical profiling of the original ryegrass plant

hosting AR153 focused on the main bioactive alkaloids: dahuelmusin A, epoxyjanthitrems, ergovaline, lolines, lolitrem B and peramine. The grass-endophyte association produced peramine, while chanoclavine was detected in the ergovaline analysis due to the higher-than-usual levels produced by this association. There were no detectable mammalian toxic compounds such as the epoxyjanthitrems, lolitrem B or ergovaline. Peramine does not cause animal toxicity (Pownall et al. 1995). Chanoclavine is the first stable intermediate in the ergot alkaloid biosynthetic pathway (Panaccione et al. 2012; Young et al. 2013) and has no toxicologically-significant effects on mammals, as assessed in a 3-week acute toxicity feeding study on mice (Finch et al. 2019). The alkaloid profiles of AR153, AR1 and SE perennial ryegrass cv. Samson were compared in a field trial at Ōhaupō with four replicates (see the Insect protection and Agronomic sections for further detail), using the method of Bastias et al. (2025). Fifty tillers per plot were cut to ground level and dead matter removed on 30 November 2023, 8 months after sowing and 9 days after grazing. The tillers were analysed for endophyte presence by immunoblot (Simpson et al. 2018) and alkaloid concentrations in the dry matter (DM). The alkaloid data were adjusted by the endophyte infection rate to provide values equivalent to 100% infection. AR153 was lower ($P < 0.05$) in peramine than AR1 and SE at 22.6 vs. 33.0 and 29.1 mg/kg DM (Figure 1A) and higher ($P < 0.05$) in chanoclavine than SE at 2.86 vs. 0.34 mg/kg DM (Figure 1B). Neither ergovaline nor lolitrem B was detected for AR153 and AR1.

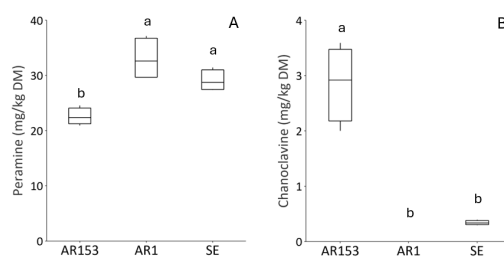


Figure 1 Peramine (A) and chanoclavine (B) concentrations for AR153, AR1 and Standard endophyte (SE) perennial ryegrass cv. Samson on 30 November 2023, 8 months after sowing, at Ōhaupō. Means with different letters are significantly different ($P < 0.05$).

AR153 and AR1 perennial ryegrass cv. Reason were compared in pot trials at Ruakura (see Root aphid section) and Lincoln (see Porina section). Twenty 10-month-old plants per treatment were harvested to ground level on 8 August 2025 at Ruakura, and five bulks of five 3-month-old plants were harvested to ground

level on 13 March 2025 at Lincoln. Untransformed peramine data were compared by one-way ANOVA in Minitab. At Ruakura, peramine concentration was not different ($P=0.286$) between AR153 and AR1 at 35.6 and 39.4 mg/kg DM, but at Lincoln it was higher ($P<0.001$) for AR153 than AR1 at 26.9 and 19.4 mg/kg DM. Ergovaline and lolitrem B were absent for both strains.

AR153, AR1 and SE perennial ryegrass cv. Samson were compared during four grazing periods in 2024 in a sheep trial at Lincoln with three replicates (see Animal health and welfare section). Untransformed data were analysed by linear regression with endophyte and date as fixed effects. Where appropriate, Robust (Huber–White) standard errors were used to account for potential heteroskedasticity. Model assumptions were assessed using residual diagnostics; the distribution of residuals was examined visually using histograms with overlaid normal densities and quantile–quantile plots. Endophyte and date effects were evaluated using Wald tests. Peramine concentration was lower ($P<0.001$) for AR153 than AR1 and SE in January–March and November–December (Table 3) and matched previous reports for grazed pastures (Tozer et al. 2024). Chanoclavine was consistently higher ($P<0.001$) for AR153 than SE and AR1 (Table 3), the latter of which does not produce chanoclavine (Moate et al. 2012), and matched previous observations for ergovaline with peak levels in summer (Thom et al. 2013; Tozer et al. 2024)."

Genome sequencing

The position of approximately 34,000,000 base pairs of the *Epichloë* AR37 reference strain genome, as described in Caradus et al. (2025), was compared to AR153 and close relatives of AR37 (AR40 and AR128) as well as distantly related strains (AR1 and AR5) using standard bioinformatic pipelines in SAMtools version 1.8 and bcftools version 1.9 (Afkhami (2012). The comparison of AR153 with AR37 identified approximately 139,000 SNPs (Single Nucleotide Polymorphisms) and 22,000 INDELs (Insertions and Deletions) (Table 1). This level of divergence was substantially greater than that observed for the closely related AR37-like strains AR40 and AR128 (Caradus et al. (2025) and similar to that observed for the distantly related strains AR1 and AR5. These results indicate that AR153 is genetically distant from AR37 and AR37-related strains and is more comparable to AR1 and AR5 in overall genome divergence. However, AR153 is genetically distinct from both AR1 and AR5, possessing thousands of variants that are absent from those strains.

Insect pest protection

Protection against major insect pests was assessed in

Table 1 Whole-genome variant comparisons relative to the AR37 reference genome.

Strain	Relationship to AR37	Approximate Number of SNPs	Approximate Number of INDELs
AR153	Study strain	139,000	22,000
AR1	Distantly related	131,000	22,000
AR5	Distantly related	134,000	22,000

a series of pot and field trials using, where applicable, protocols approved by the Endophyte Technical Committee (ETC) of the New Zealand Plant Breeding and Research Association (NZPBRA) (Caradus et al. 2021; NZPBRA 2026). Endophyte infection was confirmed on at least one tiller per plant by immunoblots (Simpson et al. 2012). Plant material for the pot trials was grown in individual 12 cm diameter (0.7 L) pots filled with commercial potting mix (Dalton’s ryegrass and clover mix) in a screenhouse under natural light and temperature conditions at Ruakura, unless otherwise stated. Plants were top-watered and fertilised with 70 mL per plant of liquid fertiliser (1.5 g/L Yates® Thrive all-purpose soluble fertiliser plus 1.3 g/L urea) as required. Significantly different means were identified using Tukey’s or Bonferroni method.

Argentine stem weevil (*Listronotus bonariensis*)

General method

Dead sheaths and tillers were removed from each plant to facilitate ASW adult feeding and the plants were trimmed to 7 cm height. Adult ASW were collected from Waikato pastures using a modified blower vac (Hewitt et al. 2025c). Six adults were caged onto each plant using fabric mesh cages fastened with elastic bands and held above the plant foliage with a wire hoop. After 2 weeks, the cages were removed and plants assessed for adult feeding damage and egg-laying. The total number of live and dead tillers per plant, number of eggs per plant and number of adult feeding scars on six randomly selected tillers per plant were counted. Adult scars were scaled with respect to a ‘standard’ small feeding scar so that larger scars could be counted as multiple scars, e.g., a hole twice as big was counted as two scars. Plants were trimmed to 5 cm (above the eggs, which are laid in the pseudostem) and returned to their original positions in the screenhouse. After a further 3–4 weeks, all tillers were cut below the meristem. The total number of live and dead tillers per plant was recorded. Up to 20 tillers per plant were inspected for ASW larval damage. The proportion of live and dead tillers assessed was the same as for the whole plant (i.e., if 90% of the tillers on a given plant were live then 18 live and two dead tillers

were assessed for that plant). Larval ASW damage was scored as: 0 = no damage, 1 = minor damage to the outside of the tiller, not penetrating to the centre, 2 = moderate damage, where the larva has penetrated and partially mined the tiller, and 3 = severe damage, where the larva has extensively mined the tiller and/or the meristem has been destroyed.

Pot trial

AR153, AR1, AR37 and Nil endophyte perennial ryegrass cv. Samson were compared in a whole-plant, no-choice pot trial with 16 replicates, as 12-month-old plants in January 2024. Adult feeding scars and egg numbers per plant were analysed using a loglink negative binomial model, with squared column position and the square root of surrounding egg numbers as covariates. Total damage (score 1, 2, 3) and moderate-severely damaged tillers (score 2 + 3) were analysed using logitlink betabinomial models. The number of adult feeding scars per tiller and number of eggs per plant were lower ($P < 0.05$) for AR153 and AR1 than Nil (Table 2). AR153 reduced the percentage of tillers with larval ASW damage by 55% and the percentage with moderate to severe larval damage by 64% compared with Nil plants ($P < 0.05$). AR153 damage was indistinguishable from AR1.

Ōhaupō field trial

AR153, AR1, AR37 and Nil endophyte perennial ryegrass cv. Samson were compared in a field trial at Ōhaupō with four replicates and 1.5 m × 4.5 m plots in January 2025. The ryegrass was sown at 20 kg/ha on 20 March 2023. Seedling emergence was high and not different across treatments on 18 April 2023 (>80% of drill rows with seedlings). Endophyte infection (immunoblot of 50 tillers per plot) was >90% for all endophytes and 1% for Nil on 30 November 2023 and ≥97% for all endophytes and 8% for Nil on 3 December 2024. Live, non-reproductive tillers were cut just below the meristem from 50 random plants from three of the four replicate plots per treatment. Adult feeding scars, eggs and larvae on each tiller were counted. Larval damage was scored as described in the pot trials. Persistence was scored as: 1 = a completely dead plot, 2 = <30% green cover to 9 = >90% coverage of plot with live/green plants, on 16 January 2025. Adult feeding scars per tiller and ground cover scores were analysed using linear mixed models fitted by restricted maximum likelihood (REML). Percentage of tillers with larval damage was analysed using generalised linear models (GLM) with a binomial distribution and logit link, while a betabinomial distribution was used for moderate-severe damage to account for overdispersion. Treatment was included as a fixed effect and block as a random effect in all models. The fixed effect was tested using Type II Wald chisquare tests, followed by unadjusted pairwise comparisons of estimated marginal

means when it was significant, consistent with Fisher's protected Least Significant Difference (LSD) approach. AR153 had lower ($P < 0.05$) levels of adult feeding and larval damage than Nil and its persistence was not different from the other treatments (Table 2).

African black beetle (*Heteronychus arator*)

General method

Adult African black beetles (ABB) were collected from Waikato pastures using pitfall traps during 2 weeks prior to each experiment. Beetles were maintained in 2 L plastic containers partially filled with soil and fed carrot segments. Only active, uninjured individuals were selected. One male and one female were caged onto each plant. Plants were assessed for adult feeding damage after 1–2 weeks. Damaged tillers were removed below the crown, and each tiller was assigned a visual damage score: 0 = no damage, 1 = minor damage – surface feeding only, 2 = moderate damage – shredding at the base of tiller or higher up, but not through whole width, tiller upright and not wilted, 3 = severe damage – tiller base shredded completely, tiller wilted or dead.

Pot Trial 1

AR153, AR1, AR37 and Nil endophyte perennial ryegrass cv. Samson were compared in a whole-plant, no-choice pot trial with 15 replicates, as 8-month-old plants in September 2021. The number of tillers per plant was modelled using a negative binomial regression model and included replicate as a factor. The percentage per plant of any tiller damage (sum of damage scores 1, 2 and 3 divided by total tillers checked) and moderate to severe damage per tiller (sum of damage scores 2 and 3 divided by total tillers checked) were modelled using a logit-link beta binomial regression model. The square root of the replicate was incorporated as an explanatory variable. AR153 had more ($P < 0.05$) adult ABB feeding damage than AR37 (Table 2).

Pot Trial 2

AR153, AR1, AR37 and Nil endophyte perennial ryegrass cv. Reason were compared in a whole-plant, no-choice pot trial with 17 replicates, as 6-month-old plants in October 2025. The percentage per plant of total tiller damage (number of tillers with damage scores 1, 2, or 3 divided by total tillers) and moderate to severe damage (number of tillers with damage scores 2 or 3 divided by total tillers) was modelled using a logit-link beta binomial regression model and replicate as an explanatory variable. AR153 reduced adult ABB feeding compared with AR1 and Nil ($P < 0.001$), with 26% fewer damaged tillers than AR1 (Table 2).

Ruakura field trial

AR153, AR1 and AR37 perennial ryegrass cv. Samson were compared in a field trial at Ruakura with three replicates and 1.5 m × 3 m plots, in February 2022. The ryegrass was sown at 20 kg/ha on 29 April 2020. Plants were sampled using a 10 × 10 cm quadrat

thrown randomly into each plot. A plant within the quadrat was gently pulled and scored on a 0–3 scale based on anchorage strength, presence of ABB larvae and/or frass and overall visual health. Ten plants per plot were scored for damage as: 0 = plant firmly anchored by roots, or plant pulled, but no evidence of ABB larval damage to the roots; 1 = slight pulling, plant loose, but still anchored by roots and evidence of ABB larval damage (frass, tunnelling, root pruning, or larva present); 2 = pulling with roots eaten and plant loose, but some healthy roots remain and evidence of ABB larval damage; 3 = complete pulling with roots fully eaten off, plant dead and evidence of ABB larval damage. The number of plants per plot with any pulling (score 1, 2 or 3) and moderate to severe pulling (score 2 or 3) was analysed as a binomial response using GLMs with a logit link and replicate as a random variable. The significance of the fixed terms was assessed using Type II (Wald) tests measured by a chi-square statistic followed by a multiple-comparison procedure based on the significance of estimated marginal mean differences. Pairwise comparisons of estimated marginal means were conducted using Tukey's multiple comparison adjustment. Pulling damage caused by ABB larval feeding was greater ($P < 0.05$) for AR153 and AR1 than AR37 (Table 2).

Ōhaupō field trial

AR153, AR1, AR37 and Nil endophyte perennial ryegrass cv. Samson were compared in the Ōhaupō field trial (ASW section) in February 2024 and 2025. ABB larval damage was assessed using the 'pull test' described for the Ruakura field trial. Plant persistence was assessed by scoring for ground cover on 2 May 2025. A visual score was made of each plot based on the approximate percentage of live/green ground cover, as: 1 = a completely dead plot, 2 = <30% green plant coverage, to 9 = >90% coverage of live plants. Counts of plants per plot with any pulling damage (scores 1–3) and moderate to severe damage (scores 2–3) were analysed as outlined for the Ruakura field trial. In February 2024, all three endophytes had lower ($P < 0.05$) pulling damage than Nil, which had moderate-severe damage (Table 2). By February 2025, only AR37 had less ($P < 0.05$) damage than Nil, and by May 2025, AR37 showed better ($P < 0.001$) persistence than all other treatments.

Root aphid (*Aploneura lentisci*)

General method

Plants were transplanted individually into 12-cm-diameter plastic pots containing a 2:1 soil:sand mixture (topsoil sourced from Captain Compost, Hamilton, New Zealand). Each plant was inoculated with mature and immature wingless root aphids using small root segments excised from root aphid-infested perennial ryegrass plants. Root segments containing

two or three mature aphids were placed into a shallow divot in the soil of each pot and gently covered with soil. Plants were inoculated a second time using the same method after a further 2 weeks. Root aphids were left undisturbed for 5–7 months to allow numbers to increase, after which root masses were washed and the aphids recovered for counting. Soil and plant roots were washed into a bucket and left for a few minutes to allow the soil to settle to the bottom and root aphids to rise to the surface. The liquid was decanted through a 300 µm sieve to catch the aphids and the retentate was carefully washed into a specimen vial with water. The washed plants were placed in individual paper bags and dried in an oven at 80°C for 48 hours. Dried roots were detached below the crown and weighed.

For root aphids counts, samples were transferred to a beaker and diluted as necessary to give a total volume of 50 or 60 mL, depending on the size of the original sample and the number of aphids present. The sample was stirred thoroughly to disperse the aphids before removing a 10 mL subsample in five 2 mL aliquots. The subsample was transferred to a 9-cm-diameter Petri dish marked with a grid (approximately 1 cm²) to facilitate counting of the aphids in the dish. Counting was carried out under a stereo microscope at 10x magnification. The number of aphids in a subsample was multiplied by five or six depending on the whole sample volume, to give the total number of root aphids per plant. The number of aphids per gram of root DM was also calculated for each plant.

Pot Trial 1

AR153, AR1, AR37 and Nil endophyte perennial ryegrass cv. Samson were compared in a whole-plant pot trial with 20 replicates. Plants were inoculated with root aphids in May 2023 and assessed at 23 months-of-age in December 2024. The number of root aphids per plant and per gram of root DM were analysed using linear mixed models estimated by REML with treatment as a fixed effect and replicate as a random effect. The fixed term was assessed using type II (Wald) tests measured by a chi-square statistic followed by a multiple-comparison procedure based on the significance of estimated marginal mean differences using a Bonferroni correction to adjust P values for multiple comparisons. Only AR37 had fewer ($P < 0.05$) root aphids and more ($P < 0.05$) root DM than other treatments (Table 2).

Pot Trial 2

AR153, AR1, AR37 and Nil endophyte perennial ryegrass cv. Samson were compared in a whole-plant pot trial with 20 replicates. Plants were inoculated with root aphids in March 2024 and assessed at 17 months-of-age in August 2025. Number of root aphids per gram of root DM was analysed as described for Pot Trial 1. AR153 and Nil had more ($P < 0.05$) root aphids

than AR1, and AR37 had fewer ($P < 0.05$) root aphids and more ($P < 0.05$) root DM than the other treatments (Table 2).

Porina (*Wiseana cervinata*)

General method

Porina eggs were collected in November 2024 from Berwick, on the Taieri Plain near Mosgiel. Female moths were caught by hand when attracted to an ultraviolet light source. The moths were held individually in 60 mL specimen containers at ambient temperature overnight during which time they laid their eggs. The species was identified by examining the bursa copulatrix of the female moth (Dugdale 1994) as identified by restriction fragment polymorphism of the mitochondrial cytochrome oxidase I gene (Richards et al. 2017). The following day, the eggs were surface sterilised by washing in 1 mg/L copper oxychloride solution for 1 minute and then rinsing with distilled water for 2 minutes (Carpenter 1983). They were then placed in groups of 100–300 eggs on moist filter paper in 9-cm-diameter Petri dishes. These were then held in darkness in a temperature-controlled room at 16–18°C. Each larva was visually inspected for injuries before each experiment. Healthy-looking larvae were weighed and assigned to each replicate, such that each larva within a replicate across the treatments had a similar weight.

Pot trial

AR153, AR1, AR37 and Nil endophyte perennial ryegrass cv. Reason were compared in a pot trial with 15 replicates at Lincoln, as 11-week-old plants in February 2025. On hatching, the larvae were moved to plastic containers with a 2–4-cm-deep layer of commercial “No 8” potting mix that had been microwaved to above 100°C to kill any invertebrates (particularly mites and fungus gnats). When cooled, the potting mix was sealed in plastic bags and stored at 4°C until used. The larvae were provided with white clover (*Trifolium repens* L.) and Nil endophyte Italian ryegrass (*L. multiflorum* Lam.) cv. Moata herbage for 14 days. Thereafter, glasshouse-grown white clover and Nil endophyte ryegrass herbage, cut into 2–3 cm sections, was laid on top of the potting mix as required until the pot trial started. A fine nylon gauze (1 mm) was placed at the bottom of each pot to prevent larvae escaping via the drainage holes, and the pots were filled to a depth of 6 cm with commercial potting mix (Daltons Seed and Cell mix). On 19 February 2025, larvae (2.5 months old, weighing between 8 and 100 mg) were weighed and then held in individual containers overnight at 18°C. The following day, one 6th instar larva was added to each plant. The trial was run for 4 weeks in a greenhouse, with overhead watering as required and supplementary light supplied by grow lights.

The number of tillers per plant was recorded at the

beginning and end of the trial. Plants were scored weekly for damage as: 0 = no damage, 1 = little bite on standing tiller, 2 = more feeding on standing tiller, 3 = chopped tiller but did not eat it (higher feeding on tiller), 4 = removed tiller and tiller is eaten at the base, 5 = only a stump is remaining. These damage scores were summed over the 4-week period for analysis. On 21 March, after 4 weeks, the trial was destructively harvested. The herbage in each pot was harvested at ground level and dried at 60°C for 12 hours before weighing to determine DM. The pots were emptied and the potting mix was searched to recover surviving larvae. Where larvae were not recovered, they were assumed to have died. The surviving larvae were weighed.

Plant damage was analysed using a GLM with a log link function. Porina larva starting weight and the square of the experimental block number were included as covariates. Treatments were compared by estimating mean numbers of damaged tillers for a hypothetical centre block with starting larval weight equal to the mean of the starting weights. Porina survival data were analysed using a binomial GLM with a logit link function. Final larval weight at the end of the trial was analysed using a multi-linear regression analysis with porina starting weight included as a covariate. Final plant dry weight was analysed using multi-linear regression analysis. Treatments were compared by assuming plant initial tillers equal to the mean of the initial tillers.

Only AR37 had lower ($P < 0.001$) porina damage and growth than other treatments (Table 2).

Bioassays

AR153, AR1, AR37 and Nil endophyte perennial ryegrass cv. Reason were compared in bioassays with 12-week-old plants and 15 replicates at Ruakura and Lincoln. Larvae were collected as described in the general methods and reared at Ruakura as described in Hewitt et al. (2025b), or at Lincoln as described above. The bioassays were conducted in controlled environment rooms at 18°C with a 16:8 hours light: dark regime from 11 February until 5 March 2025 (3 weeks). Larvae were 2.5 months old (29–165 mg) at Ruakura and 2 months old (8–100 mg) at Lincoln and were starved overnight prior to the experiments. Individual larvae were placed into plastic specimen containers (70 mL) three-quarters filled with fine bark chips (Garden Highlights, 15–25 mm). These containers were placed into polystyrene trays and loosely covered with black polythene excluding light to minimise the degradation of fungal alkaloids.

Semi-synthetic diets, that had freeze-dried, ground pseudostem plant material incorporated, were prepared following the protocol of Hewitt et al. (2025b). Diets were prepared weekly for 3 weeks. Plugs (180 mg $\pm$ 20

mg) of 12 mm diameter were extracted from the set diet using a cork borer. At each diet change, larvae were provided with one semi-synthetic diet plug from the relevant treatment. Diets were changed in each larval container on days 4 and 7 of each week. Diets were stored at 4°C between changes.

Consumption was assessed as the difference in diet wet weight between changes. Daily diet consumption was calculated by dividing the diet weight change

by the number of days between diet changes. Larval weights were recorded at the end of the 3-week trial, allowing the calculation of their growth.

Data from Ruakura and Lincoln were pooled following inspection, as both experiments showed the same response patterns. All variables were analysed using linear mixed effects models, with endophyte strain as a fixed effect and initial larval weight as a random effect. Larvae that died during the bioassays were excluded

Table 2 Bioactivity of AR153, AR1, AR37 and Nil endophyte perennial ryegrass against Argentine stem weevil, African black beetle, root aphid and porina in pot trials, field trials and bioassays. Within rows, means with different letters are significantly different ($P < 0.05$).

Insect and Trial	Measured Trait	Endophyte Treatment				
		AR153	AR1	AR37	Nil	P Value
Argentine stem weevil						
Pot trial	Adult feeding scars (n/tiller)	10.7 ab	8.7 a	16.6 bc	19.2 c	<0.001
	Eggs per plant	1.1 a	2.5 ab	7.3 bc	9.7 c	<0.001
	% moderate-severe larval damage/tiller	11 a	11 a	10 a	30 b	0.01
	% total larval damage	17 a	23 ab	29 ab	38 b	0.06
Ōhaupō field trial	Adult feeding scars (n/tiller)	1.0 a	1.0 a	1.5 a	3.2 b	<0.001
	% tillers per plot with moderate-severe larval damage	11.3 a	10.7 a	14.1 a	37.3 b	<0.001
	% tillers per plot with any larval damage	26.7 a	28.0 a	28.0 a	64.0 b	<0.001
	Persistence score January 2025	4.8 ab	4.2 ab	5.7 a	3.0 b	0.022
African black beetle						
Pot Trial 1	% moderate-severely damaged tillers per plant	58 b	42 b	27 a	53 b	<0.001
	% damaged tillers per plant	60 b	44 ab	28 a	57 b	<0.001
Pot Trial 2	% moderate-severely damaged tillers per plant	43 b	67 c	21 a	90 d	<0.001
	% damaged tillers per plant	43 b	69 c	21 a	91 d	<0.001
Ruakura field trial	% plants with moderate-severe pulling	67 b	63 b	23 a	-	0.002
	% plants with any pulling	70 b	63 b	23 a	-	0.001
Ōhaupō field trial	% plants with moderate-severe pulling February 2024	23 a	25 a	8 a	80 b	<0.001
	% plants with any pulling February 2024	23 a	28 a	20 a	88 b	<0.001
	% plants with moderate-severe pulling February 2025	68 ab	60 ab	40 a	78 b	0.007
	% plants with any pulling February 2025	68 ab	68 ab	45 a	78 b	0.02
	Persistence score May 2025	1.0 b	1.2 b	5.3 a	1.0 b	<0.001
Root aphid						
Pot Trial 1	Number of root aphids/g root dry matter	150 b	118 b	3 a	89 b	<0.001
	Root dry weight (g/plant)	0.5 b	0.4 b	0.7 a	0.5 b	<0.001
Pot Trial 2	Number of root aphids/g root dry matter	512 c	212 b	13 a	449 c	<0.001
	Root dry matter (g/plant)	1.1 a	1.2 a	2.2 b	1.0 a	<0.001
Porina						
Pot trial	Severely damaged or dead tillers (score 3–5)	6.7 b	7.7 b	2.0 a	6.4 b	<0.001
	Plant weight (g dry matter/plant)	7.5	7.9	8.2	8.2	NS
	% larval survival	59	87	62	72	NS
	Larval weight (mg)	276 b	241 b	161 a	270 b	<0.001
Bioassays	Daily larval consumption (mg)	53.8 b	53.4 b	15.4 a	56.9 b	<0.001
	Larval weight gain (mg)	90.8 b	93.1 b	4.6 a	101.1 b	<0.001
	% larval survival	90.1 b	93.4 b	46 a	83.5 b	<0.001

from the weight gain analysis.

Only AR37 had lower ($P<0.001$) porina feeding, growth and survival than other treatments (Table 2).

Insect Pest Conclusion

AR153 demonstrated strong bioactivity against both adult and larval ASW, comparable to the highly deterrent AR1, and low bioactivity against ABB, root aphid and porina, similar to AR1.

Animal health and welfare

Animal safety was assessed for AR153, AR1, SE and Nil endophyte perennial ryegrass cv. Samson using sheep during five grazing periods (January–March 2021 and January–March, May, September–October and November–December in 2024) at Lincoln, following methods in Fletcher et al. (2017) and approved by the ETC of the NZPBRA (Caradus et al. 2021). All animal experimentation was approved by AgResearch Animal Ethics Committee.

Pastures

Three replicates of each treatment were sown in a randomised block design with 0.175 ha plots on 14–22 September 2020 and 3–7 October 2023. Irrigation, nitrogen fertiliser and herbicide applications supported growth of the ryegrass monocultures. Pastures were topped on 30 December 2020, 19 January 2021 and 8 January 2024. Tiller endophyte infection was measured prior to the January–March grazing each year by immunoblot (Simpson et al. 2012). DNA analysis of these tillers in 2024, using high-resolution melt (Gagic et al. 2018), confirmed endophyte strain. Compressed pasture height, measured with a rising plate meter prior to and during grazing periods, was used to estimate pasture mass and equalise allocation per sheep utilising a put-and-take system. Herbage samples were cut to ground level from each plot at the beginning, mid-point and end of each grazing period. These were analysed for peramine, ergovaline, lolitrem B and chanoclavine using methods in Bastías et al. (2025).

Animals

Weaned 4-month-old Romney ewe lambs were purchased in December each year. Sheep were under common management prior to and between the grazing periods. During these non-measurement periods, sheep grazed endophyte-free grass and clover mixtures or lucerne (*Medicago sativa* L.) monocultures. Sheep were vaccinated for clostridial disease, drenched with anthelmintics and dipped to prevent flystrike (myiasis), in line with best-management farm practice. At the beginning of each grazing period, ten core sheep were allocated to each plot such that mean initial live weights across treatments were not different. The sheep grazed for 42 days in January–March 2021 and 40, 28, 30 and 33 days in January–March, May, September–October

and November–December in 2024, respectively.

The animal variables measured were live weight at the start, mid-point and end of each grazing period, dag accumulation (score 0–5) at the mid-point and end of each grazing period, and ryegrass staggers (RGS) at 2–3-day intervals following the first symptom being detected. RGS scoring used the methods in Keogh (1973), with the exception that sheep developing a score of 4 were removed from the trial to ensure that the RGS could not get any higher. These sheep were replaced with spare sheep, but their score (4) was retained for calculation of treatment means. To determine tolerance to heat load, the sheep were temporarily moved into a purpose-built tunnel house with an ambient temperature of 30–40°C during February 2021, February 2024 and December 2024, and their rectal temperature and respiration rate were measured. Sheep were observed at least twice daily for general health and well-being.

RGS data were analysed with a linear mixed effects model in R version 4.4.1 with ‘predictmeans’ and ‘lme4’ packages. For liveweight gain (LWG), respiration rate and rectal temperature, the 2021 data were analysed by ANOVA in Genstat 18th edition and the 2024 data were analysed with a mixed effects model in R version 4.3.3 with ‘predictmeans’ and ‘lme4’ packages.

Results

Mean endophyte infection of tillers for AR153, AR1 and SE was 98%, 94% and 94% in 2021 and 99%, 92% and 89% in 2024, respectively. This was above the required 85% threshold for ETC animal trials. The Nil treatment had 1% endophyte infection in 2021 and 0% in 2024. In 2024, AR37 contamination was measured in the AR153 (1%) and SE (2%) treatments, and AR1 contamination in the SE (1%) treatment.

No endophyte alkaloids were detected for Nil ryegrass. AR153, AR1 and SE contained peramine with the concentrations being highest in January–March 2024 and lowest in September–October 2024 (Table 3). Both AR153 and SE contained chanoclavine with AR153 having the highest concentration. Only SE contained ergovaline and lolitrem B, which reached highest concentrations in January–March.

SE had a RGS score of 2.3 after 16 days during January–March 2021 and 2.1 after 19 days in 2024 (Figure 2), which exceeded the ETC threshold for a valid test. In both grazing periods, RGS scores of <1.5 were detected for AR1 after 29–35 days and no RGS were detected for AR153 and Nil. No RGS occurred in May and September–October and RGS was only detected for SE in November–December (0.3 after 33 days).

Sheep grazing SE had a higher ($P<0.05$) mean rectal temperature and respiration rate in February 2021 and 2024 and a higher ($P<0.05$) respiration rate in December

Table 3 Mean concentrations of peramine, ergovaline, lolitrem B and chanoclavine for AR153, AR1 and Standard endophyte (SE) perennial ryegrass cv. Samson during five grazing periods in 2021 and 2024 at Lincoln.

Grazing Period and Treatment	Alkaloid Concentration (mg/kg DM)			
	Peramine	Ergovaline	LolitreB	Chanoclavine
January–March 2021				
AR153	15.2	0.0	0.0	1.8
AR1	21.8	0.0	0.0	0.0
SE	21.7	0.9	2.2	0.3
January–March 2024				
AR153	20.0	0.0	0.0	1.7
AR1	28.1	0.0	0.0	0.0
SE	27.4	1.0	2.5	0.3
May 2024				
AR153	17.6	0.0	0.0	0.8
AR1	19.9	0.0	0.0	0.0
SE	19.1	0.4	1.2	0.1
September–October 2024				
AR153	8.0	0.0	0.0	0.7
AR1	8.6	0.0	0.0	0.0
SE	6.4	0.4	0.3	0.0
November–December 2024				
AR153	13.5	0.0	0.0	1.3
AR1	15.9	0.0	0.0	0.0
SE	16.7	0.5	1.4	0.1

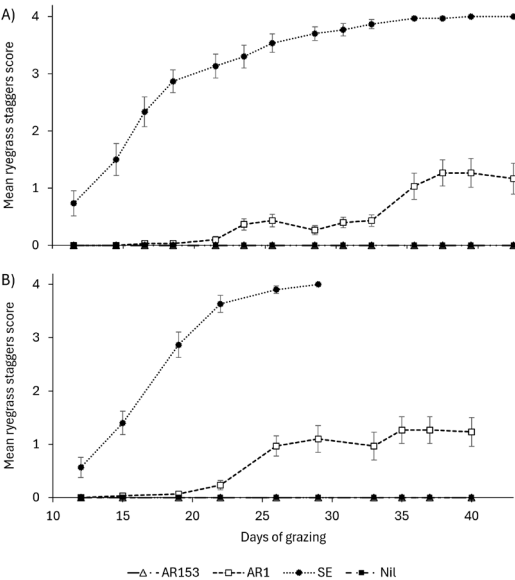


Figure 2 Ryegrass staggers score (mean $\pm$ standard error) of sheep grazing AR153, AR1, Standard (SE), or Nil endophyte perennial ryegrass cv. Samson during January–March in 2021 (A) and 2024 (B) at Lincoln.

2024 than all other treatments, which were not different to each other (Table 4).

Liveweight gain was lower ($P<0.05$) for SE than all other treatments, which were not different to each other, during January–March 2021 and 2024 (Table 5). In the second half of both periods, no sheep with a RGS score of <4 remained on the SE treatment by day 39 in 2021 and day 29 in 2024, therefore no liveweight gain was recorded. Liveweight gain was lower ($P<0.05$) for SE than AR153 and AR1 during the first half of the May 2024 grazing period and lower ($P<0.05$) for AR1 and SE than Nil during the second half.

Dag accumulation was below score 0.3 and not different across all treatments in January–March 2021, January–March 2024 and May 2024. It averaged 1.2 for SE compared with <0.4 ($P<0.001$) for the other treatments in September–October 2024, and 0.6 for SE compared with 0.1 ($P<0.01$) for the other treatments in November–December 2024.

Animal Health and Welfare Conclusion

No negative animal health and welfare issues were

Table 4 Rectal temperature and respiration rate (mean ± standard error) of sheep grazing AR153, AR1, Standard (SE), or Nil endophyte perennial ryegrass cv. Samson while under heat load in February 2021, February 2024 and December 2024 at Lincoln. Within columns and months, means with different letters are significantly different (P<0.05).

Month and Treatment	Rectal Temperature (°C)	Respiration Rate (Breaths/Minute)
February 2021		
AR153	40.44 ± 0.08 b	178 ± 2.0 b
AR1	40.36 ± 0.07 b	174 ± 2.6 b
SE	40.69 ± 0.11 a	194 ± 2.0 a
Nil	40.38 ± 0.06 b	172 ± 2.2 b
P Value	0.011	<0.001
February 2024		
AR153	40.06 ± 0.08 b	215 ± 8.7 b
AR1	40.15 ± 0.07 b	223 ± 8.7 b
SE	40.47 ± 0.08 a	277 ± 9.0 a
Nil	40.03 ± 0.08 b	220 ± 8.8 b
P Value	≤0.030	<0.006
December 2024		
AR153	39.99 ± 0.14	176 ± 7.9 b
AR1	40.07 ± 0.14	188 ± 7.8 b
SE	40.29 ± 0.14	215 ± 7.8 a
Nil	40.09 ± 0.14	179 ± 7.9 b
P Value	NS	<0.001

NS = not significant.

Table 5 Liveweight gain (LWG) from start to mid-trial, and from mid to end of trial (mean ± standard error), of sheep grazing AR153, AR1, Standard (SE), or Nil endophyte perennial ryegrass cv. Samson during the first and second halves of January–March 2021 and 2024, and May 2024 grazing periods at Lincoln. Within columns and grazing periods, means with different letters are significantly different (P<0.05).

Grazing Period and Endophyte	LWG in First Half (g/day)	LWG in Second Half (g/day)
January–March 2021		
AR153	271 ± 36.6 a	-22 ± 64.4
AR1	286 ± 34.5 a	-19 ± 60.6
SE	102 ± 34.4 b	-*
Nil	241 ± 34.2 a	14 ± 60.0
P Value	0.023	NS
January–March 2024		
AR153	266 ± 29.6 a	68 ± 43.8
AR1	245 ± 29.6 a	78 ± 43.8
SE	135 ± 29.6 b	-
Nil	295 ± 29.6 a	48 ± 43.8
P Value	<0.001	NS
May 2024		
AR153	103 ± 16.8 a	29 ± 17.4 ab
AR1	95 ± 16.8 a	-7 ± 17.4 b
SE	45 ± 16.8 b	-20 ± 17.4 b
Nil	78 ± 16.8 ab	50 ± 17.4 a
P Value	<0.050	<0.050

*No sheep remained on SE at the final weigh date. NS = not significant.

observed in sheep grazing AR153-infected ryegrass, whereas low levels of RGS were observed for AR1. This suggests that AR153 lacks one or more factors present in AR1 that may contribute to mild RGS, potentially including differences in alkaloid concentrations or other endophyte-derived metabolites.

Food safety

Some metabolites produced by endophytes have previously been shown to be present in the milk (Finch et al. 2013) and fat (Finch et al. 2012) of animals grazing endophyte-infected pastures. For this reason, it is reasonable to assume that any novel compounds produced by a new endophyte have the potential to end up in the human food chain. A food safety trial using mice fed a diet containing endophyte-infected seed allows an assessment of whether any compound produced by the endophyte poses a potential risk to human consumers.

Sub-acute toxicity was determined by feeding groups of mice (five male and five female, 3-weeks old) diets based on normal rodent chow containing either: 1) 30% AR153-infected seed, 2) no seed, or 3) 30% Nil endophyte seed, for 21 days using a well-established protocol (Finch et al. 2021). Mice were individually caged and diet weighed in and out to allow daily food consumption to be measured. Body weight was determined daily and blood pressure, heart rate and grip strength weekly. After 21 days, mice were euthanised and a blood sample taken for measurement of plasma enzymes and haematological parameters. Major organs were weighed and these organs, along with additional tissue samples, were prepared for histopathological examination. All animal experimentation was approved by AgResearch Animal Ethics Committee.

There were no differences between mice fed Nil or AR153 seed, including blood chemistry, haematology, organ weight, heart rate, blood pressure, grip strength and histopathology, which revealed no adverse effects of AR153.

Seed transmission and storage viability

The transmission of AR153 over successive seed crops was examined in perennial ryegrass cv. Samson (diploid), Reason (diploid), Align (tetraploid) and Italian ryegrass cv. Manta (diploid) between 2018 and 2026 at Lincoln. The initial plants were inoculated with AR153. Seed was harvested over three generations for Samson, two for Reason and Align and one for Manta. For Samson and Reason, each successive crop used seed from the previous harvest, with no reselection between seed generations to improve endophyte infection. For Align, the second crop was sown with seed from plants that transmitted endophyte at the first

harvest. The harvested seed was cleaned and stored at 0–3°C and 30% relative humidity. The numbers of initial plants and seeds from each harvest with viable AR153 were tested by immunoblot and RNA high-

Table 6 Year of first seed crop, number of initial plants infected with AR153 and percentage of seeds with viable AR153 over successive seed crops for perennial ryegrass cv. Samson, Reason and Align and Italian ryegrass cv. Manta.

Trait	Cultivar				
	Seed Crop	Samson	Reason	Align	Manta
Year of first seed crop		2019	2024	2025	2025
Number of initial plants infected with AR153		30	52	47	42
% seeds with AR153	First	99.0 ^a	100 for 51 plants, 0 for one plant ^b	99.6 ^a	99.5 for 40 plants, 0 for two plants
	Second	98.5	99.8 ^c		100 ^c
	Third	98.9			

^a All plants transmitted endophyte to seed.
^b Post-harvest testing showed this plant was endophyte-free.
^c Second seed crop was sown with seed from plants that transmitted endophyte at the first seed harvest.

resolution melt methods (Gagic et al. 2018). The seeds were tested by growing seedlings and testing one tiller per seedling. For the first crop, up to 16 seedlings from each inoculated plant were tested for Samson, Reason and Align and 24 seedlings for Manta. For the second and third crops, 100–364 seedlings were tested per cultivar. Transmission levels were high and showed no or very little decline over each seed generation (Table 6).

The percentage of perennial ryegrass cv. Samson seeds with AR153 was compared with AR1 over 23 months of storage at ambient temperature and humidity. This comparison was part of a larger experiment reported in Caradus et al. (2025). Two seed lots per strain and eight paper packets per seed lot, each with approximately 2.5 g of seed, were stored from April 2021 to March 2023 at Kimihia (near Lincoln). At about 3-month intervals, a packet from each seed lot was removed and the percentage of seeds with viable endophyte was tested by growing 100 seedlings and testing these by immunoblot (Simpson et al. 2012). The percentage data and 84.3% confidence intervals were transformed for each timepoint onto a logit scale,

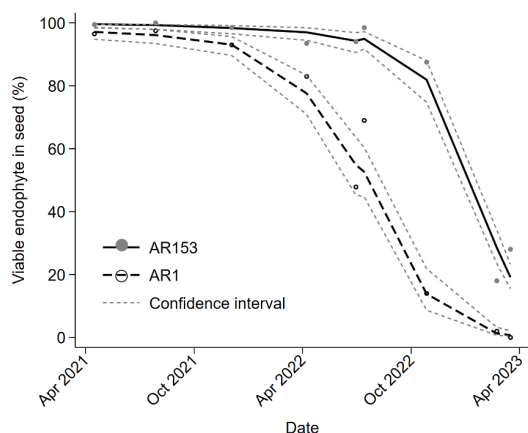


Figure 3 Percentage (mean $\pm$ 83.4% confidence interval) of perennial ryegrass cv. Samson seeds with viable AR153 and AR1 over 23 months of storage at ambient temperature and humidity. Symbols indicate actual values.

smoothed using LOWESS and then back transformed. The data were then analysed for effects of strain, timepoint and their interaction by GLM with a binomial function and robust variance in Stata SE version 18. The percentage of seeds with viable AR153 declined less rapidly ($P < 0.001$) than AR1 during storage (Figure 3).

Agronomic robustness

Dry matter yield and persistence were examined at Ruakura, Ōhaupō and Kimihia Research Centre. In each trial, ryegrass was sown into a cultivated seedbed at 20 kg/ha using a small-plot cone seeder at 10–15 mm depth and 15 cm row spacing. Plots were either mown or grazed by sheep or cattle (depending on the objectives and practical constraints of each trial) when pasture mass reached 2,000–2,500 kg DM/ha. In the grazed trials, plots were mown post-grazing to give equal residuals when necessary. Soil fertility was maintained at optimal levels, e.g. pH no less than 5.8 and Olsen P no less than 25 mg/L, with regular soil tests and fertilisation. N fertiliser was applied at approximately 360 kg N/ha/year for mown trials and 120–220 kg N/ha/year for grazed trials. Herbicides were applied if needed to control white clover, grass weeds (e.g., *Digitaria sanguinalis* (L.) Scop.) and broadleaf weeds.

At Ruakura and Ōhaupō, DM yield was measured by cutting a 0.45 m-wide strip along the length of each plot to a height of 4 cm using a rotary mower. At Kimihia, the entire plot was harvested to a height of 5 cm using a Haldrup forage harvester. At Ruakura, ryegrass persistence was visually scored as: 1 = a completely dead plot, 2 = <30% green cover, to 9 = >90% green

cover. The number and timing of yield and persistence assessments are presented with the results for each trial. In some cases, measurements only occurred when endophyte treatment differences were visually apparent. An optimal REML spatial model was fitted to each trial measurement in Genstat 24. The model with the minimal Bayesian Information Coefficient (Gilmour et al. 1997) was selected as the optimal model.

Ruakura field trial

AR153, AR1, AR37 and SE perennial ryegrass cv. Samson were compared at 17 harvests over 2 years (2020/21 and 2021/22) at the Ruakura field trial described in the ABB section. Results are shown for seasons when treatment effects occurred and in total for Year 2. AR153 yield was less ($P < 0.05$) than AR37 and SE in autumn of Year 1, greater ($P < 0.05$) than AR1 in summer and less ($P < 0.05$) than AR37 and SE in autumn of Year 2, and less ($P < 0.05$) than AR37 and SE for the whole of Year 2 (Table 7). Severe ABB larval damage (Table 2) reduced persistence more ($P < 0.05$) for AR153 and AR1 than AR37 and SE in autumn of Year 2.

Kimihia field trial

AR153, AR1, AR37 and SE perennial ryegrass cv. Samson were compared over 3 years (2020/21 to 2023/24) at Kimihia with four replicates and 1.5 m $\times$ 4.5 m plots. The ryegrass was sown on 5 March 2020 and harvested 28 times. On average across years, AR153 yield was lower ($P < 0.05$) than AR37 and SE in winter and AR1 in spring, but not different than all other treatments for the rest of the year and in total (Table 8).

Ōhaupō field trial

AR153, AR1, AR37, SE and Nil endophyte perennial ryegrass cv. Samson were compared in 2024 and 2025 at the Ōhaupō field trial described in the ASW section. Persistence was visually scored as: 1 = completely dead, 2 = <30% green cover, to 9 = >90% green cover. After the ABB larval damage in February 2024 (Table 2), all endophyte treatments were more ($P < 0.05$) persistent than Nil until November 2024 (Figure 4). After the ASW and ABB larval damage in January–February 2025 (Table 2), AR37 and SE were more ($P < 0.05$) persistent than AR153 and AR1, which both declined to be no different to Nil by May 2025.

Second Ōhaupō and Kimihia field trials

AR153 and AR1 perennial ryegrass cv. Samson and Reason were compared over 2 years (2024/25 and 2025/26) in a second field trial at Ōhaupō and Kimihia, each with four replicates and 1.5 m $\times$ 4.5 m plots. The ryegrass was sown at 20 kg/ha on 26 March 2024 at Ōhaupō and 8 April 2024 at Kimihia and harvested 18 times. At Ōhaupō, AR153 yield (averaged across years) was greater ($P < 0.05$) than AR1 for cv. Reason in late spring, greater ($P < 0.05$) than AR1 for both cultivars in summer and 7–9% greater ($P < 0.05$) than AR1 for both

Table 7 Dry matter yield and persistence of AR153, AR1, AR37 and Standard endophyte (SE) perennial ryegrass cv. Samson over 2 years (2020/21 and 2021/22) after sowing in autumn 2020 at Ruakura, for two seasons when treatment effects occurred and in total for Year 2. Within columns, means with different letters are significantly different ($P<0.05$).

Endophyte	Yield (kg DM/ha)				Persistence Score*
	Autumn Year 1	Summer Year 2	Autumn Year 2	Total Year 2	Autumn Year 2
AR153	1,728 b	696 a	41 b	6,650 b	2.7 b
AR1	1,495 b	448 b	0 b	6,776 b	2.1 b
AR37	2,230 a	924 a	433 a	8,050 a	4.9 a
SE	2,294 a	913 a	594 a	8,221 a	5.5 a
P Value	<0.001	<0.001	<0.001	<0.001	<0.001

* 1 = completely dead, 2 = <30% cover to 9 = >90% cover.

Table 8 Dry matter yield of AR153, AR1, AR37 and Standard endophyte (SE) perennial ryegrass cv. Samson over 3 years (2020/21 to 2023/24) after sowing in autumn 2020 at Kimihia, for five seasons and annually, averaged across years. Within columns, means with different letters are significantly different ($P<0.05$).

Endophyte	Yield (kg DM/ha)					
	Winter	Spring	Late Spring	Summer	Autumn	Total
AR153	1,433 b	2,817 b	3,916	2,930	1,714	12,810
AR1	1,494 ab	3,019 a	3,968	2,982	1,749	13,212
AR37	1,527 a	2,804 b	4,048	2,983	1,839	13,201
SE	1,455 a	2,872 ab	3,817	2,914	1,621	12,679
P Value	<0.001	<0.001	NS	NS	NS	NS

NS = not significant.

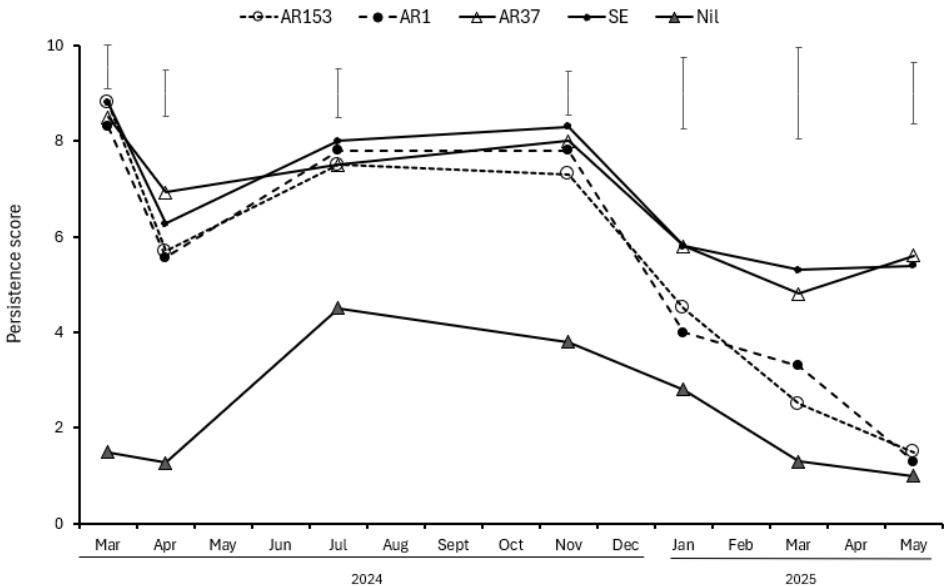


Figure 4 Persistence score (1 = completely dead, 2 = <30% green cover to 9 = >90% green cover) of AR153, AR1, AR37, Standard (SE) and il endophyte perennial ryegrass cv. Samson in 2024 and 2025 after sowing in autumn 2023 at Ōhau. Error bars represent the least significant difference ($P<0.05$).

Table 9 Dry matter yield of AR153 and AR1 perennial ryegrass cv. Samson and Reason averaged over 2 years (2024/25 and 2025/26) after sowing in autumn 2024 at Ōhaupō and Kimihia, for five seasons and annually, averaged across years. Within columns and sites, means with different letters are significantly different ($P<0.05$).

Site and Treatment	Yield (kg DM/ha)					
	Winter	Spring	Late Spring	Summer	Autumn	Total
Ōhaupō						
AR153 Samson	930 bc	2,156	2,831 c	2,482 ab	1,621	10,020 c
AR1 Samson	850 c	1,967	2,719 c	2,181 c	1,629	9,346 d
AR153 Reason	1,066 a	2,197	3,637 a	2,603 a	1,731	11,234 a
AR1 Reason	1,015 ab	2,169	3,393 b	2,340 bc	1,759	10,676 b
P Value	<0.05	NS	<0.001	<0.05	NS	<0.001
Kimihia						
AR153 Samson	689 c	3,183	4,415 b	4,545	2,817 ab	15,649 b
AR1 Samson	681 c	3,208	4,602 a	4,493	2,749 b	15,733 b
AR153 Reason	736 b	3,196	4,616 a	4,786	3,027 a	16,361 a
AR1 Reason	789 a	3,225	4,584 a	4,684	3,027 a	16,309 a
P Value	<0.001	NS	<0.05	NS	<0.05	<0.05

NS = not significant.

cultivars in total (Table 9). At Kimihia, AR153 yield was lower ($P<0.05$) than AR1 for cv. Reason in winter and for cv. Samson in late spring, but not different to AR1 in total.

Agronomic Robustness Conclusion

Overall, ryegrasses cultivars infected with AR153 and AR1 had similar agronomic performance as measured by yield and persistence over the five trials. In the Waikato trials, when insect pressure was high, both these endophytes had poorer yields and persistence than AR37 and SE.

Discussion

Four decades of research on *Epichloë* endophytes in ryegrass in New Zealand has transformed pasture systems through the identification and deployment of selected endophyte strains. These endophytes enhance plant resistance to key insect pests while minimising the mammalian toxic effects historically associated with SE. As a result, they support improved animal health and welfare and contribute to greater pastoral productivity, including increased meat and milk yields relative to toxic SE associations. Today, the widespread adoption of these selected *Epichloë* strains has resulted in their incorporation into many proprietary perennial ryegrass cultivars used in New Zealand’s pastoral agriculture (Caradus 2023; Hewitt et al. 2025a).

The results of the present study show that AR153 occupies a similar functional niche to AR1 within the spectrum of commercially available endophytes. Both endophytes provided strong protection against ASW,

which was expected given the presence of peramine, a key determinant of ASW resistance (Rowan et al. 1990; Popay et al. 2003). AR153 and AR1 share a similar alkaloid profile, with the principal difference being the production of chanoclavine by AR153. Chanoclavine is an intermediate in the ergot alkaloid biosynthetic pathway, and studies of pathway mutants have provided a framework for investigating the ecological significance of individual alkaloids within this pathway (Fleetwood et al. 2007). Although the specific biological role of chanoclavine remains unclear, its presence may contribute to differences between AR153 and AR1. However, the current study was not designed to isolate the effects of chanoclavine, and further research would be required to determine whether it contributes directly to insect resistance, pasture persistence, or other agronomically important traits.

Protection against ASW remains highly relevant to New Zealand pasture systems, as damage during pasture establishment can substantially reduce long-term pasture performance (Popay & Thom 2009). However, as with AR1, AR153 did not provide effective control of ABB, root aphid, or porina. Therefore, while AR153 offers advantages in other traits, its insect protection profile remains broadly similar to AR1 and does not extend to the wider spectrum of pest protection provided by endophytes such as AR37.

A noteworthy finding of this study was the favourable animal-safety profile of AR153. While AR1 is widely considered animal-safe, occasional low-level RGS have been reported under some grazing conditions despite the absence of lolitrem B (Fletcher 1999). The

biological basis for these observations remains unclear. AR153 is genetically distinct from AR1. The absence of RGS observed in sheep grazing AR153 suggests that the compound/s associated with the occasional neurological responses observed for AR1 may either be absent or produced at lower concentrations in AR153.

High rates of endophyte transmission are essential for the successful utilisation of novel *Epichloë* endophytes. Previous research has shown that the agronomic benefits of endophyte infection, including improved insect resistance and pasture persistence, depend on maintaining a high frequency of infected plants within the pasture (Hume & Sewell 2014). Across multiple cultivars and two ryegrass species, AR153 consistently demonstrated very high transmission rates (>98%), indicating that endophyte infection levels can be maintained through successive generations of seed multiplication.

Maintaining high endophyte viability during storage is a key determinant of the proportion of infected plants established at sowing, and reductions in viability can compromise the persistence and productivity benefits associated with endophyte infection (Hume & Sewell 2014). The storage studies demonstrated an advantage of AR153 over AR1 in maintaining viable endophyte during ambient storage. AR153 retained commercially relevant infection levels for substantially longer than AR1. Consequently, a greater proportion of seedlings established from stored AR153 seed would be expected to contain a viable endophyte and therefore express the associated insect-resistance traits. This finding is particularly relevant given that endophyte frequency within a pasture is positively associated with the expression of endophyte-mediated benefits (Hume & Sewell 2014).

Collectively, these results demonstrate that AR153 combines strong protection against ASW with a favourable animal-safety profile, high transmission rates and improved storage stability. While AR153 does not provide the broader pest protection spectrum associated with endophytes such as AR37, the results highlight the continued opportunity to refine endophyte technology through the selection of strains that optimise both pasture performance and livestock wellbeing.

Practical implications

AR153 exhibited similar efficacy to AR1 against the major target pests and comparable agronomic performance, while demonstrating an absence of RGS, reliable endophyte transmission to seed and improved seed storage characteristics. Collectively, these findings indicate that AR153 retains the key biological attributes of AR1 while addressing some of its limitations, supporting its suitability as a successor endophyte for ryegrass pastures where ABB, root aphid and porina

pressure is low.

ACKNOWLEDGEMENTS

To Wei Zhang for preparing AR153 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; Colin Ferguson for collection of porina eggs; Shery Hanna, Sarah Jackman and Scott Hardwick for running the Lincoln insect trials; 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* strains marketed under the brands AR1®, AR37®, AR128®, AR153®, 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®, AR153®, MaxQ®, MaxQII®, MaxP®, MaxR®, Avanex®, Happe® and Protek®. David Hume, Alison Popay and Wade Mace are inventors on the AR128® and AR153® patent.

REFERENCES

- Afkhami ME. 2012. Fungal endophyte-grass symbioses are rare in the California floristic province and other regions with Mediterranean-influenced climates. *Fungal Ecology* 5: 345-352. <https://doi.org/10.1016/j.funeco.2011.09.006>
- Bastías DA, Johnson LJ, Jáuregui R, Applegate ER, Liu X, Mace WJ, Card SD. 2025. *Paenibacillus taichungensis* strain E222: Mother and progeny plant growth promotion and association with an *Epichloë* fungal endophyte. *Plant Physiology and Biochemistry*: 110851. <https://doi.org/10.1016/j.plaphy.2025.110851>
- Caradus JR, Lovatt S, Belgrave B. 2013. Adoption of forage technologies. *Proceedings of the New Zealand Grassland Association* 75: <https://doi.org/10.33584/jnzg.2013.75.2917>
- Caradus JR, Johnson LJ. 2020. *Epichloë* fungal endophytes—From a biological curiosity in wild grasses to an essential component of resilient high performing ryegrass and fescue pastures. *Journal of Fungi* 6: 322. <https://doi.org/10.3390/jof6040322>
- Caradus JR, Chapman D, Cookson T, Cotching B, Deighton M, Donnelly L, Ferguson J, Finch S, Gard S, Hume D. 2021. *Epichloë* endophytes—new perspectives on a key ingredient for resilient perennial grass pastures. *Resilient Pastures Symposium*:

- Grassland Research and Practice Series 17: Hamilton, New Zealand. <https://doi.org/10.33584/rps.17.2021.3435>
- Caradus JR. 2023. *Epichloë* fungal endophytes—a vital component for perennial ryegrass survival in New Zealand. *New Zealand Journal of Agricultural Research*: 1-18. <https://doi.org/10.1080/00288233.2023.2170426>
- Caradus JR, Hume D, Popay A, de Bonth A, Croy R, Forester T, George R, Rosanowski S, Maclean P, Mace W, Faville MJ, Norriss MG, Maw B, Hewitt KG, Jensen JG, Finch SC. 2025. AR128—a new *Epichloë* fungal endophyte product for ryegrass. *Proceedings of the 11th International Symposium of Grass Microbial Endophytes: Grassland Research and Practice Series 18*: 151-169. <https://doi.org/10.33584/rps.18.2025.3771>
- Card SD, Bastias DA, Zhang W, Hume DE, Caradus JR. 2024. *Epichloë*—a key element of New Zealand's agricultural landscape. *New Zealand Journal of Botany* 62: 519-547. <https://doi.org/10.1080/0028825X.2024.2331570>
- Carpenter A. 1983. Chemical treatment of porina eggs to prevent loss of viability in culture. *New Zealand Entomologist* 7: 466-467. <https://doi.org/10.1080/00779962.1983.9722443>
- Christensen MJ, Bennett RJ, Schmid J. 2002. Growth of *Epichloë/Neotyphodium* and p-endophytes in leaves of *Lolium* and *Festuca* grasses. *Mycological Research* 106: 93-106. <https://doi.org/10.1017/S095375620100510X>
- Di Menna M, Finch S, Popay A, Smith B. 2012. A review of the *Neotyphodium lolii/Lolium perenne* symbiosis and its associated effects on animal and plant health, with particular emphasis on ryegrass staggers. *New Zealand Veterinary Journal* 60: 315-328. <https://doi.org/10.1080/00480169.2012.697429>
- Dugdale JS. 1994. Hepialidae (insecta: lepidoptera). *Fauna of New Zealand* 30. <https://doi.org/10.7931/J2/FNZ.30>
- Easton HS, Christensen MJ, Eerens JPJ, Fletcher LR, Hume DE, Keogh RG, Lane GA, Latch GCM, Pennell CGL, Popay AJ, Rolston MP, Sutherland BL, Tapper BA. 2001. Ryegrass endophyte: a New Zealand Grassland success story. *Proceedings of the New Zealand Grassland Association* 63: <https://doi.org/10.33584/jnrg.2001.63.2429>
- Finch SC, Fletcher LR, Babu JV. 2012. The evaluation of endophyte toxin residues in sheep fat. *New Zealand Veterinary Journal* 60: 56-60. <https://doi.org/10.1080/00480169.2011.634746>
- Finch SC, Thom E, Babu J, Hawkes A, Waugh C. 2013. The evaluation of fungal endophyte toxin residues in milk. *New Zealand Veterinary Journal* 61: 11-17. <https://doi.org/10.1080/00480169.2012.704626>
- Finch SC, Munday JS, Sprosen JM, Bhattarai S. 2019. Toxicity studies of chanoclavine in mice. *Toxins* 11: 249. <https://doi.org/10.3390/toxins11050249>
- Finch SC, Webb NG, Boundy MJ, Harwood DT, Munday JS, Sprosen JM, Cave VM, Broadhurst RB, Nicolas J. 2021. Sub-acute feeding study of saxitoxin to mice confirms the effectiveness of current regulatory limits for paralytic shellfish toxins. *Toxins* 13: 627. <https://doi.org/10.3390/toxins13090627>
- Fleetwood DJ, Scott B, Lane GA, Tanaka A, Johnson RD. 2007. A complex ergovaline gene cluster in *Epichloë* endophytes of grasses. *Applied and Environmental Microbiology* 73: 2571-2579. <https://doi.org/10.1128/AEM.00257-07>
- Fletcher LR, Sutherland BL, Fletcher CG. 1999. The impact of endophyte on the health and productivity of sheep grazing ryegrass based pastures. *Ryegrass Endophyte - An Essential New Zealand Symbiosis. Grassland Research and Practice Series 7*: <https://doi.org/10.33584/rps.7.1999.3403>
- Fletcher LR. 1999. "Non-toxic" endophytes in ryegrass and their effect on livestock health and production. *Ryegrass Endophyte - An Essential New Zealand Symbiosis Grassland Research and Practice Series 7*: <https://doi.org/10.33584/rps.7.1999.3393>
- Fletcher LR, Finch SC, Sutherland BL, deNicolo G, Mace WJ, Van Koten C, Hume DE. 2017. The occurrence of ryegrass staggers and heat stress in sheep grazing ryegrass-endophyte associations with diverse alkaloid profiles. *New Zealand Veterinary Journal* 65: 232-241. <https://doi.org/10.1080/00480169.2017.1329673>
- Gagic M, Faville MJ, Zhang W, Forester NT, Rolston MP, Johnson RD, Ganesh S, Koolaard JP, Easton H, Hudson D. 2018. Seed transmission of *Epichloë* endophytes in *Lolium perenne* is heavily influenced by host genetics. *Frontiers in Plant Science* 9: 1580. <https://doi.org/10.3389/fpls.2018.01580>
- Gilmour AR, Cullis BR, Verbyla AP. 1997. Accounting for natural and extraneous variation in the analysis of field experiments. *Journal of Agricultural, Biological, and Environmental Statistics*: 269-293. <https://doi.org/10.2307/1400446>
- Hewitt KG, Hofmann R, Ball O, Finch S, Bryant R, Popay A. 2025a. New Zealand's pastoral ecosystems: challenges, solutions, and the protective role of *Epichloë* endophytes. *Proceedings of the 11th International Symposium of Grass Microbial Endophytes: Grassland Research and Practice Series 18*: 29-44. <https://doi.org/10.33584/rps.18.2025.3761>
- Hewitt KG, Hofmann RW, Ball JP, Finch SC, Bryant RH, Popay AJ. 2025b. Phosphorus induced changes in food quality enhance porina fitness feeding on *Epichloë* endophyte free forage grasses. *Scientific Reports* 15. <https://doi.org/10.1038/s41598-025->

89723-5

- Hewitt KG, Hofmann RW, Ball OJ, Somchit C, Ferguson CM, Moss RJ, Bryant RH, Popay AJ. 2025c. Influence of phosphorus fertiliser use on two New Zealand pasture insect pest communities *Agriculture, Ecosystems & Environment* 394. <https://doi.org/10.1016/j.agee.2025.109878>
- Hume D, Sewell J. 2014. Agronomic advantages conferred by endophyte infection of perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) in Australia. *Crop and Pasture Science* 65: 747-757. <https://doi.org/10.1071/CP13383>
- Johnson LJ, De Bonth ACM, Briggs LR, Caradus JR, Finch SC, Fleetwood DJ, Fletcher LR, Hume DE, Johnson RD, Popay AJ, Tapper BA, Simpson WR, Voisey CR, Card SD. 2013. The exploitation of *epichloae* endophytes for agricultural benefit. *Fungal Diversity* 60: 171-188. <http://doi.org/10.1007/s13225-013-0239-4>
- Keogh R. 1973. Induction and prevention of ryegrass staggers in grazing sheep. *New Zealand Journal of Experimental Agriculture* 1: 5-57. <https://doi.org/10.1080/03015521.1973.10427616>
- Moate PJ, Williams SRO, Grainger C, Hannah MC, Mapleson D, Auldred MJ, Greenwood JS, Popay AJ, Hume DE, Mace WJ, Wales WJ. 2012. Effects of wild-type, AR1 and AR37 endophyte-infected perennial ryegrass on dairy production in Victoria, Australia. *Animal Production Science* 52: 1117-1130. <https://doi.org/10.1071/AN12126>
- NZPBRA. 2026. *Endophyte Insect Control Ryegrass, Festulolium & Continental Tall Fescue*. Retrieved 23rd April <https://www.pbra.co.nz/scientific-papers/nzpbra-agreed-statement-on-epichloe-endophyte-viability-testing/>.
- Panaccione DG, Ryan KL, Schardl CL, Florea S. 2012. Analysis and modification of ergot alkaloid profiles in fungi. *Methods in Enzymology*, pp. 267-290. Elsevier. <https://doi.org/10.1016/B978-0-12-394290-6.00012-4>
- Pennell CGL, Popay AJ, Ball OJ-P, Hume DE, Baird DB. 2005. Occurrence and impact of pasture mealybug (*Balanococcus poae*) and root aphid (*Aploneura lentisci*) on ryegrass (*Lolium* spp.) with and without infection by *Neotyphodium* fungal endophytes. *New Zealand Journal of Agricultural Research* 48: 329-337. <https://doi.org/10.1080/00288233.2005.9513663>
- Popay AJ, Hume DE, Davies KL, Tapper BA. 2003. Interactions between endophyte (*Neotyphodium* spp.) and ploidy in hybrid and perennial ryegrass cultivars and their effects on Argentine stem weevil (*Listronotus bonariensis*). *New Zealand Journal of Agricultural Research* 46: 311-319. <https://doi.org/10.1080/00288233.2003.9513559>
- Popay AJ, Thom ER. 2009. Endophyte effects on major insect pests in Waikato dairy pasture. *Journal of New Zealand Grasslands* 71: <https://doi.org/10.33584/jnzg.2009.71.2758>
- Pownall DB, Familton AS, Field RJ, Fletcher LR, Lane GA. 1995. The effect of peramine ingestion in penned lambs. *Proceedings of the New Zealand Society of Animal Production* 55:186.
- Realini FM, Escobedo VM, Ueno AC, Bastías DA, Schardl CL, Biganzoli F, Gundel PE. 2024. Anti-herbivory defences delivered by *Epichloë* fungal endophytes: a quantitative review of alkaloid concentration variation among hosts and plant parts. *Annals of Botany* 133: 509-520. <https://doi.org/10.1093/aob/mcae014>
- Richards NK, Mansfield S, Townsend RJ, Ferguson CM. 2017. Genetic variation within species and haplotypes of the *Wiseana* (Lepidoptera: Hepialidae) complex and development of non-sequenced based identification tools to aid field studies. *Pest Management Science* 73: 2334-2344. <https://doi.org/10.1002/ps.4620>
- Rowan DD, Dymock JJ, Brimble MA. 1990. Effect of fungal metabolite peramine and analogs on feeding and development of Argentine stem weevil (*Listronotus bonariensis*). *Journal of Chemical Ecology* 16: 1683-1695. <https://doi.org/10.1007/BF01014100>
- Ruppert KG, Matthew C, McKenzie CM, Popay AJ. 2017. Impact of *Epichloë* endophytes on adult Argentine stem weevil damage to perennial ryegrass seedlings. *Entomologia Experimentalis et Applicata* 163: 328-337. <https://doi.org/10.1111/eea.12584>
- Schardl CL, Young CA, Faulkner JR, Florea S, Pan J. 2012. Chemotypic diversity of *epichloae*, fungal symbionts of grasses. *Fungal Ecology* 5: 331-344. <https://doi.org/10.1016/j.funeco.2011.04.005>
- Simpson WR, Schmid J, Singh J, Faville MJ, Johnson RD. 2012. A morphological change in the fungal symbiont *Neotyphodium lolii* induces dwarfing in its host plant *Lolium perenne*. *Fungal Biology* 116: 234-240. <https://doi.org/10.1016/j.funbio.2011.11.006>
- Thom ER, Popay AJ, Waugh CD, Minneé EMK. 2013. Impact of novel endophytes in perennial ryegrass on herbage production and insect pests from pastures under dairy cow grazing in northern New Zealand. *Grass and Forage Science* 69: 191-204. <http://doi.org/10.1111/gfs.12040>
- Tozer KN, Hume DE, Cameron C, Greenfield R, Dale T, Mace WJ, Craven T, Faville MJ. 2024. Effects of heading date and *Epichloë* endophyte on persistence of diploid perennial ryegrass (*Lolium perenne*). 2. Endophyte strain and interactions with heading date. *Crop & Pasture Science* 75: CP24101. <https://doi.org/10.1080/00288233.2003.9513559>

- [org/10.1071/CP24101](https://doi.org/10.1071/CP24101)
Young CA, Hume DE, McCulley RL. 2013. Forages and pastures symposium: Fungal endophytes of tall fescue and perennial ryegrass: Pasture friend or foe? *Journal of Animal Science* 91: 2379-2394. <https://doi.org/10.2527/jas.2012-5951>
- Zhang W, Forester NT, Moon CD, Maclean PH, Gagic M, Arojju SK, Card SD, Matthew C, Johnson RD, Johnson LJ. 2022. *Epichloë* seed transmission efficiency is influenced by plant defense response mechanisms. *Frontiers in Plant Science* 13: 1025698. <https://doi.org/10.3389/fpls.2022.1025698>