Impact of selected *Epichloë* endophytes on perennial ryegrass yield performance across regions in New Zealand

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Abstract

Over the past couple of decades selected *Epichloë* endophytes have been vital to farmers and the economy in New Zealand, with AR1 and AR37 being two of the frontier and foundational ryegrass endophyte strains. These endophytes produce different alkaloid profiles which defines the scope of pest resistance afforded by them to the host grass and suitability for use in different areas of New Zealand. Data collected by the New Zealand Plant Breeding and Research Association as a part of the National Forage Variety Trials provide information to understand the yield advantages that endophyte can have in regions with different insect and environmental limitations. The analysis focused on the impact of AR37 and AR1 on a diploid perennial ryegrass cultivar (cv. Ceres ONE^{50}) evaluated in 20 trials throughout New Zealand.

There were significant regional and seasonal endophyte effects, with the greatest benefits being consistent with when insect and abiotic pressures were expected to be the greatest. AR37 had the broadest level of increased yield while AR1 still offered yield advantages over endophyte-free plants in many cases. Dry matter yield did not differ between AR37 and AR1 at a national level except for autumn, or regionally except for the Upper North Island. The benefit of AR37 was seen to the greatest extent in the Upper North Island, with a 33% and 51% yield advantage over AR1 and endophyte-free, respectively. For the Upper South Island both AR37 and AR1 gave a significant increase in DM yield over endophyte-free plants for annual dry matter yield.

There were individual trials in which AR1-infected and endophyte-free plants gave inadequate protection, resulting in pasture failure, others in which insect pests or other stressors are likely to have impacted yield without causing pasture failure, and some where there was little differentiation between plants infected with selected endophyte strains and those that were endophyte-free. This highlights the wide variation that can be observed on individual farms across New Zealand and the role that selected endophytes play in creating resilient pastures.

Keywords: AR1, AR37, insect pests, resilience

Introduction

New Zealand's intensively managed agriculture systems are based on pastoral species, largely perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), introduced in the 19th century. These pasture species have not evolved natural resistance against the array of insect pests that now occur in New Zealand. In New Zealand pastures, both exotic and native insects build to population densities not seen in the more complex ecosystems elsewhere in the world (Goldson et al. 2020).

Meeting the demand for on-farm feed requires a consistent flow of pasture-grown dry matter (DM) throughout the year with varying seasonal requirements. With perennial ryegrass DM yields being a key driver of on-farm performance, plant breeders are continually striving to provide new perennial ryegrass cultivars with increased seasonal and annual yield performance (Caradus et al. 2021a; Easton et al. 2002a), in association with selected *Epichloë* endophytes that are appropriate for the region (Caradus 2024; Popay and Hume 2011).

Major forage insect pests in New Zealand include Argentine stem weevil (*Listronotus bonariensis*), pasture mealybug (*Balanococcus poae*), African black beetle (*Heteronychus arator*), root aphid (*Aploneura lentisci*), porina (*Wiseana* spp.) and the New Zealand grass grub (*Costelytra giveni*) which can each cause loss of production or even pasture failure when present at high population densities. The economic impact in New Zealand from damage caused by Argentine stem weevil has been estimated at NZ$200 million/year, African black beetle at NZ$242 million/year, grass grub

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Table 1 Insect resistance in diploid perennial ryegrasses, either endophyte-free or infected with AR37 or AR1 (adapted from NZPBRA 2024).

Endophyte status	Argentine stem weevil	Pasture mealybug	Black beetle	Root aphid	Porina	Grass grub
AR37	++++[1]	++++	+++	++++	+++	+
AR1	++++	++++	+	-[2]	-	-
Endophyte-free	-	-	-	-	-	-

- No bioactivity
+ Low level of bioactivity
++ Moderate bioactivity
+++ Good bioactivity
++++ Very good bioactivity

[1] AR37 exhibits bioactivity towards Argentine stem weevil larvae but not adults

[2] AR1 infected plants are more susceptible to root aphid than endophyte-free plants

between NZ$215–585 million/year and porina up to NZ$170 million/year (Caradus et al. 2021b; Ferguson et al. 2018). Damage from insect pests may reduce pasture yields even if populations are insufficient to lead to plant death, and selected *Epichloë* strains have been a key tool for the protection against insect pests in established pastures (Popay and Hume 2011). As a result of their alkaloid profiles, the selected endophytes AR1, and AR37, were commercially offered in New Zealand due to their resistance to common insect pests (Table 1) whilst addressing some of the animal health issues seen with the consumption by grazing livestock of toxic alkaloids associated with the common-toxic endophyte strain (Fletcher 2005; Caradus et al. 2021a). There are four main chemical classes of secondary metabolite produced by *Epichloë* spp.: indole diterpenes (e.g. lolitrem B and epoxyjanthitrems), ergot alkaloids (e.g. ergovaline), pyrrolizidines (e.g. lolines) and the pyrrolopyrazines (e.g. peramine) (Lane 1999). Of the endophytes in this study, AR37 produces high concentrations of epoxyjanthitrems and AR1 produces high levels of peramine, while endophyte-free ryegrass plants do not produce any of these secondary metabolites (Caradus et al. 2021a).

Selected endophytes, such as AR1 and AR37, are now used in a wide range of commercially available ryegrass cultivars in New Zealand and have contributed significantly to the economy. AR37 has been estimated to have contributed NZ$3.6 billion since its release 20 years ago (ACIL Allen Consulting, 2017). Due to the differences in pest protection afforded by each selected endophyte, comprehensive regional testing of DM yield is required for each new cultivar × endophyte combination to assess productive performance within and across regions in New Zealand.

Materials and Methods

The National Forage Variety Trials (NFVT) have been operating since 1991 through the New Zealand Plant Breeding and Research Association (NZPBRA) for the evaluation of pre-commercial and commercial forage grasses. The trials are overseen and audited by members of the NZPBRA to ensure they conform to common trial protocols. Locations of the NFVT are divided into four regions in New Zealand; Upper North Island, Lower North Island, Upper South Island and Lower South Island. The NZPBRA produce summaries annually to provide cultivar DM yield performance for each region as well as National and South of Taupo (New Zealand excluding the Upper North Island) summaries.

The perennial ryegrass trials consist of pure-ryegrass-sward replicated small plots (8-16 m^2 per plot) that are measured for DM yield for 3 years on a rotation length that simulates typical on-farm grazing cycles. DM yield (measured as kg DM/ha) is determined by collecting and weighing a known area via mowing, and a subset of this dried to determine DM percentage. Post yield measurement, the remaining material is either removed by mowing (mow trial) or grazed by either sheep or cows and mown to a common residual if required. The data from each DM yield measurement is allocated into seasonal growth according to five seasons in a year: winter (June-July), early spring (August-September), late spring (October-November), summer (December-February) and autumn (March-May). Trials were scored annually for sward integrity, and any cultivar x endophyte combination which dropped below 40% of sown grass had the DM yield discontinued (subsequently recorded as 0 yield).

NFVT dataset and analysis

A total of 20 trials, undertaken between 2009 and 2024, included the diploid perennial ryegrass cv. Ceres ONE^{50}, either endophyte-free (E-) or containing the selected endophyte strains AR1 or AR37 (Table 2). Other trials that did not contain all three of these endophyte options were excluded from the analysis.

The yields were linearly interpolated to seasonal totals by allocating a proportion of each cut to seasons that were part of the cut's growth period. The proportion

Table 2 Summary of 20 National Forage Variety Trial sites for four regions in New Zealand.

Trial code	Year sown	Location	Altitude a.s.l. (m)	Mean annual rainfall (mm)	Defoliation management
		Upper North Island			
P213NST	2013	Hamilton, Waikato	40	909	Cows
P217TEA	2017	Te Awamutu, Waikato	61	1123	Cows
P218CAM	2018	Cambridge, Waikato	46	1180	Cows
P221HAM	2021	Hamilton, Waikato	40	1123	Cows
P221WHA	2021	Whangarei, Northland	20	1289	Mow
		Lower North Island			
P213MAS	2013	Palmerston North, Manawatu	60	987	Cows
P217MAR	2017	Marton, Rangitikei	128	876	Sheep
P218MAS	2018	Palmerston North, Manawatu	60	987	Cows
P219MAS	2019	Palmerston North, Manawatu	58	987	Cows
P220MAR	2020	Marton, Rangitikei	128	874	Cows
P221MAS	2021	Palmerston North, Manawatu	67	987	Cows
		Upper South Island			
P209WOD	2009	Oxford, Canterbury	300	805	Mow
P213KIM	2013	Lincoln, Canterbury	60	660*	Mow
P217KIR	2017	Kirwee, Canterbury	163	680	Mow
P218BUR	2018	Burnham, Canterbury	64	600*	Mow
P219BUR	2019	Burnham, Canterbury	65	650*	Mow
P220YAL	2020	Yaldhurst, Canterbury	50	572*	Sheep
		Lower South Island			
P214WDL	2014	Woodlands, Southland	45	1045	Sheep
P218WIN	2018	Winton, Southland	38	959	Cows
P220SUT	2020	Winton, Southland	56	1200	Mow

*Trial had irrigation in addition to rainfall

was the days of growth in that season divided by the total number of days growth in the cut i.e. the cut date – the previous cut date. Any missing values in cuts were estimated using the optimal model described below for the individual cuts, so that seasonal totals could be calculated for all plots. The seasonal totals were added within a year to give an annual total. Establishment was not included in the yield contributing to the year 1 annual yield. The seasonal and annual totals for each year were then averaged over the 3 years of the trial. These seasonal and annual summaries were calculated separately for each plot. Means and standard errors for each trial and season were found by fitting an optimal REML spatial model to the plot data using Genstat 24 (VSN International, 2024). The model with the minimal Bayesian Information Coefficient (Gilmour et at. 1997) was selected as the optimal model. The trials were combined in a meta-analysis using the method of Madden et al. (2016) using Genstat 24.

Results

National

The seasonal summary analysis of all 20 trials represents the mean yield over 3 years at a national level for season and year (annual), with all trials completed at the end of the third autumn. The dataset showed yield differences between endophytes beginning in the late spring and carrying through to autumn (Figure 1). No differences were observed between plots with AR37 and AR1 for late spring, summer and annual yield but plots with AR37 were significantly greater than E- in these periods. In contrast, yield from plots with AR37

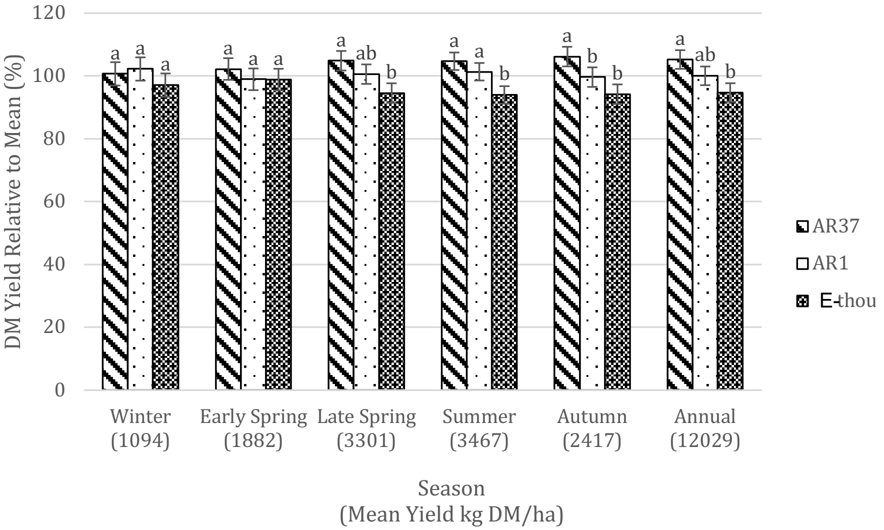

Figure 1 Mean DM yield for each season and annual total for all regions of New Zealand measured for 3 years (20 trials). Error bars show least significant interval. Columns followed by the same letter were not significantly (P>0.05) different.

were significantly greater to AR1 (6%) and E- (13%) in the autumn. The winter and early spring periods did not display an endophyte effect (Figure 1).

There were few instances of pasture loss to an extent where pasture renewal would be required if the trial plots had been sown at paddock scale for commercial use. At the end of the trial periods, only 3 of the 20 trials in this study had lower than 40% sward integrity in any of the ONE^{50} endophyte options, all located in the Upper North Island. The trials located in the other regions of New Zealand all maintained greater than 40% sward integrity during the 3 year trial duration in the entries analysed for this study.

Upper North Island region

Of the four regions, the Upper North Island had the greatest variation in endophyte effect on DM yield (Figure 2). Plots with AR37 had a yield advantage over E- in all seasons except for winter, resulting in a 51% advantage for total annual DM yield. AR37 also had a yield advantage over AR1 in the summer and autumn which resulted in 33% greater total annual yield.

Plots with AR1 did not differ from E- in any of the seasons in the combined analysis (Figure 2), however AR1 had greater total yield than E- in one of the five trials (P217TEA) where E- plants did not persist for the full 3-year trial length while plants with AR1 did. One other trial in the dataset (P218CAM) also displayed complete pasture failure in the AR1 and E- plots prior to the completion of the trial. In a third Upper North Island trial (P213NST), the E- entry was below 40% sward integrity at the completion of the trial and would have had DM yields cease if the trial had continued.

In the Upper North Island, the greatest differences between plots with selected endophyte and E- were observed in the third autumn season, just prior to trial completion. Year 3 autumn included five Upper North Island trials, four of which plots with AR37 had significantly greater yield than E- plots, three in which AR37 had significantly greater yield than AR1 and two in which AR1 had significantly greater yield than E- (Table 3).

Lower North Island region

In the Lower North Island, there were very few differences between plots in the combined 3 year seasonal and annual analysis for DM yield (Figure 3). It was only in summer that a difference was observed, where both AR37 and AR1 had 10% greater DM yield than E-. The DM yield of plots with AR37 and AR1 did not differ from each other in any season in the Lower North Island.

Given that the summer seasonal result was significant for selected endophytes compared with E-, the third-year summer was studied at an individual trial level comprising of six trials, in three of which plots with AR37 and AR1 each showed a significantly greater DM production than E- (Table 4).

Upper South Island region

In the six Upper South Island trials, plots with AR37 and AR1 had greater DM yields on average than E-

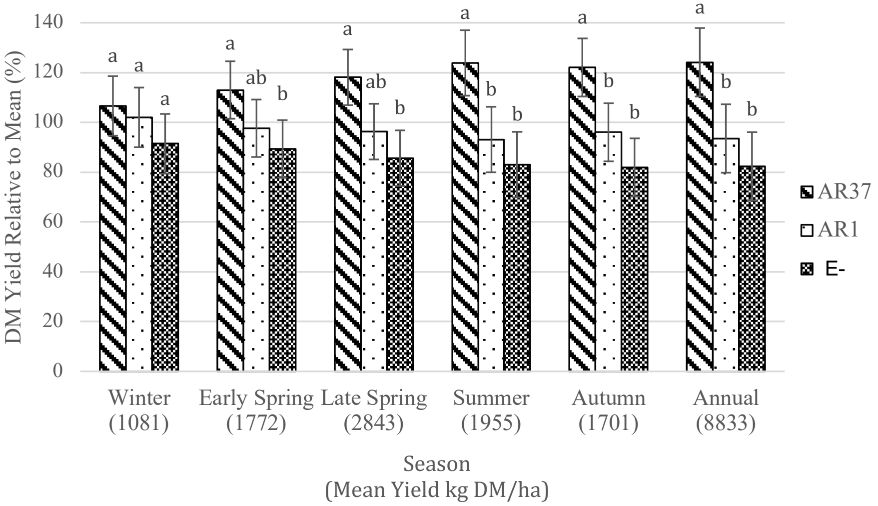

Figure 2 Upper North Island mean seasonal and annual DM yields measured for 3 years (5 trials). Error bars show least significant interval. Columns followed by the same letter were not significantly (P>0.05) different.

Table 3 Effect of endophyte on mean yields and treatment differences (kg DM/ha) in the autumn of year 3 of five trials in the Upper North Island. Bolded values indicate significant differences at P=0.05.

Trial	**DM yield (kg DM/ha)**				
	Mean yield	**AR37-E- difference**	**AR1-E- difference**	**AR37-AR1 difference**	**LSD 5%**
P213NST	798	**592**	**250**	**342**	231
P217TEA	490	**827**	**643**	183	232
P218CAM	703	**2110**	0	**2110**	281
P221HAM	3422	27	338	-311	469
P221WHA	2248	**1005**	-12	**1018**	314
Proportion of trials with significant differences		4/5	2/5	3/5	

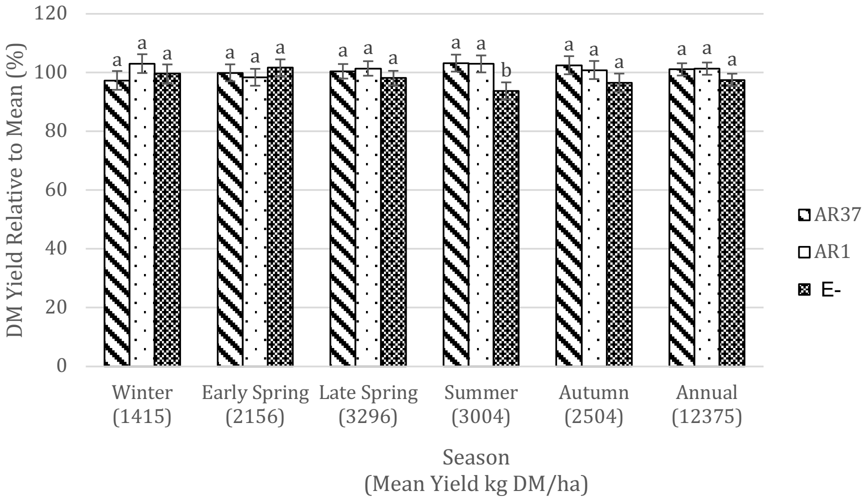

Figure 3 Lower North Island mean seasonal and annual DM yields measured for 3 years (6 trials). Error bars show least significant interval. Columns followed by the same letter were not significantly (P>0.05) different.

Table 4 Effect of endophyte on mean yields and treatment differences (kg DM/ha) in the summer of year 3 of six trials in the Lower North Island. Bolded values indicate significant differences at P=0.05.

Trial	**DM yield (kg DM/ha)**				
	Mean yield	**AR37-E- difference**	**AR1-E- difference**	**AR37-AR1 difference**	**LSD 5%**
P213MAS	2500	19	149	-131	224
P217MAR	2078	**552**	253	299	335
P218MAS	2842	-44	28	-72	382
P219MAS	2323	**511**	**325**	187	203
P220MAR	5006	**1275**	**1276**	-1	686
P221MAS	2742	18	**283**	-265	283
Proportion of trials with significant differences		3/6	3/6	0/6	

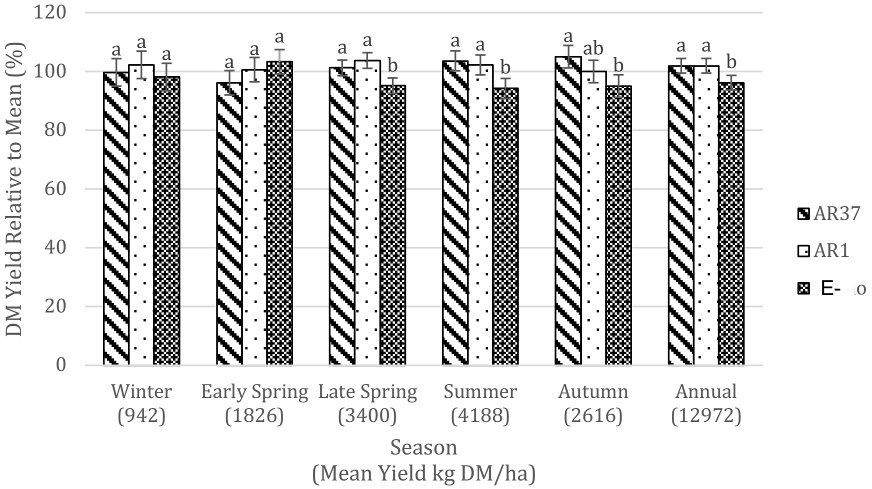

Figure 4 Upper South Island mean seasonal and annual DM yields measured for 3 years (6 trials). Error bars show least significant interval. Columns followed by the same letter were not significantly (P>0.05) different.

in the late spring and summer, with AR37 also being significantly higher in the autumn relative to E- to give a 6% greater total annual yield over E- for each AR37 and AR1 (Figure 4). The DM yields of plots with AR37 and AR1 did not differ from each other in any of the seasons in the seasonal analysis of the Upper South Island trials.

Based on the mean summer result for the Upper South Island, the third-year summer was studied at an individual trial level (Table 5). Of the six trials, there were two where AR37 yielded significantly more than E-, one trial where AR1 yielded significantly more than E-, two trials where AR37 yielded significantly more than AR1 and one trial where AR1 yielded significantly more than AR37 during the third summer.

Lower South Island Region

In the Lower South Island, the seasonal summary only had a significant difference in the late spring where AR37 had a 6% higher DM yield than E-, with AR1 not significantly different from either AR37 or E- (Figure 5). For comparison with the Lower North Island and Upper South Island, the third summer was looked at from an individual trial level where there were no differences in DM yield production between any of the endophytes in this third summer (Table 6).

Discussion

Host ryegrass variety can in itself affect the outcomes associated with different selected endophytes, with alkaloid expression having the ability to differ between cultivars (Easton et al. 2002b). Limiting this study to just one cultivar provides the most comparable dataset between endophyte options, but care should be taken when extrapolating to other ryegrass varieties. The results confirm that DM production, both seasonally

Table 5 Effect of endophyte on mean yields and treatment differences (kg DM/ha) in the summer of year 3 of six trials in the Upper South Island. Bolded values indicate significant differences at P=0.05.

	DM yield (kg DM/ha)				
Trial	**Mean**	**AR37- E- difference**	**AR1-E- difference**	**AR37-AR1 difference**	**LSD 5%**
P209WOD	5474	-36	**1089**	**-1125**	509
P213KIM	3243	**1423**	293	**1130**	642
P217KIR	2368	119	223	-104	323
P218BUR	5206	**1320**	444	**875**	500
P219BUR	4068	394	240	154	613
P220YAL	3263	-55	-10	-45	389
Proportion of trials with significant differences		2/6	1/6	2/6 AR37> 1/6 AR1>	

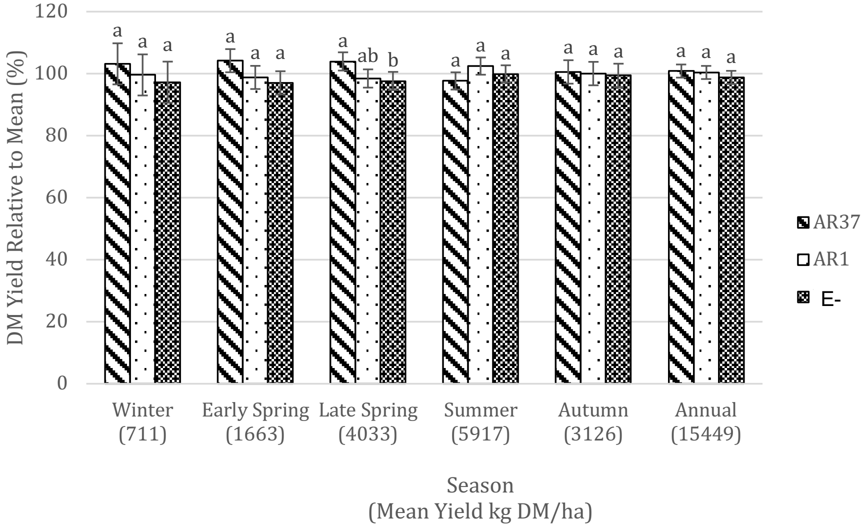

Figure 5 Lower South Island mean seasonal and annual DM yields measured for 3 years (3 trials). Error bars showing least significant interval. Columns followed by the same letter were not significantly (P>0.05) different.

Table 6 Effect of endophyte on mean yields and treatment differences (kg DM/ha) in the summer of year 3 of three trials in the Lower South Island.

	DM yield (kg DM/ha)				
Trial	**Mean**	**AR37-E- difference**	**AR1-E- difference**	**AR37-AR1 difference**	**LSD 5%**
P214WDL	8689	443	458	-14	570
P218WIN	3516	-186	362	-548	684
P220SUT	6485	-242	-375	134	619
Proportion of trials with significant differences		0/3	0/3	0/3	

and annually, needs to be considered at a regional level to understand the performance of a perennial ryegrass cultivar that has different endophyte options. The greatest differences in DM yield between plots infected with selected endophyte combinations were found in the Upper North Island where AR37 was significantly better than E- in every season except for winter, and significantly better than AR1 in the summer and autumn. The other notable difference in the Upper North Island was the consistency of a positive increase in yield for plots with AR37, with 80% and 60% of trials showing a significant DM yield advantage in the third autumn over E- and AR1, respectively. Hume et al (2007) found that in the cultivar ‘Grasslands Samson’, AR37 infected plants produced 16% greater total DM yield in a trial located in Hamilton, and 47% greater total DM yield in a trial located in Kerikeri compared to AR1. The results of the current study are in line with this previous work, with the 5 trials located in the Upper North Island having on average a 33% DM yield advantage from plots with AR37 compared with AR1.

In other regions the differences in DM yield were smaller, with the Upper South Island showing a significant difference with selected endophytes out-yielding E- plots for seasonal production between late spring through to autumn and for annual DM production, and the Lower North Island only seeing a positive DM production increase in summer with selected endophyte relative to E-. Despite these smaller differences compared to the Upper North Island, in the Lower North Island both AR37 and AR1 in the third summer showed a significant yield advantage over E- in 50% of the trials, and in the Upper South Island AR37 had significantly higher DM yield than E- in 33% of trials in the third summer. In a previous study by Hume et al (2004), within the cultivar ‘Grasslands Nui’, plots with AR37 had a greater DM yield than plots with AR1 in 45% of the summer periods and 63% of the autumn periods, while also never being lower yielding than AR1 during these seasons. There was a much smaller difference between AR37 and AR1 found in the current study in regions outside of the Upper North Island, while advantages over E- were observed.

These results show selected endophytes would be recommended in the Lower North Island and the Upper South Island in addition to the clear requisite in the Upper North Island. Strongest validation for the use of AR37 was observed in the Upper North Island but within every region there was a significant seasonal effect relative to E-. In contrast, the yield from plots with AR37 were significantly higher than the yield from plots with AR1 in the Upper North Island but the same as AR1 (on average) in other regions. The yield from plots with AR1 was the same as E- plots in the Upper North Island.

Ryegrass pests of significance include Argentine stem weevil, pasture mealybug, root aphid, porina and grass grub which are common throughout New Zealand, and African black beetle which is primarily found in the Upper North Island. With changing climate, the adoption of endophytes which provide protection to a wide variety of insects (and alkaloid concentrations sufficient to deter those insects) could increase beyond current levels of use due to an increase in pest population density and regional adaptation of pests. AR37 is recognised as providing a greater level of insect resistance than either AR1 or E- (Table 1). These results highlight, from an on-farm perspective, the choice of endophyte x cultivar combination needs to be considered from a risk management point of view. In many of the results, plots with AR1 matched the DM yield of plots with AR37, and more work is required to validate how this relates to on-farm conditions so that the appropriate suitability of endophyte selection can be made. The 3-year length of these NFVTs allow the trials to draw out relative differences for cultivar and cultivar by endophyte combinations but does give a limited timeframe for biotic and abiotic stressors to differentiate between varieties and endophytes. Given the complexity of factors contributing to pasture decline, and the results shown, the use of selected endophytes such as AR37 (containing epoxyjanthitrems) and AR1 (containing peramine) would seem applicable across New Zealand, with selection based on anticipated insect pest species and if stock suitability allows. Over time the insect species or population densities present in a region could change, as well as the abiotic stressors, and further separation in pasture yield between endophyte options may become more evident in regions of New Zealand. Currently, endophyte product choice is particularly important for the Upper North Island where endophytes which express high levels of epoxyjanthitrems can continue to provide pasture production compared to E- or endophytes that give inadequate protection to the local insect pests such as AR1. Selected endophytes should be considered a tool to moderate the impact of insect pests at a paddock scale, but consideration must also be given to suitability of selected endophytes for different livestock types based on industry recommendations for animal health and welfare (Caradus et al. 2021a).

A strict protocol is followed by the operator when a NFVT is undertaken. This enables current and new genetics to be evaluated in a consistent and repeatable process. To ensure consistency of methodology across trial sites, each ‘cultivar × endophyte’ entry is treated equally including time of DM measurement, starting from a common residual after mowing or grazing, soil fertility, fertilisation and weed control. This mitigates many of the factors that can affect differences in cultivar × endophyte DM performance that may be observed under non-controlled situations. Thus, differences in DM performance observed in the current study both within and between regions for selected endophytes and non-endophyte are likely to reflect differences in inter-regional trial sites. Whilst there is consistency in trial protocol, variability between trials for specific cultivar × endophyte combinations may still occur due to site location, climatic variation and whether pest populations are present. Further, some of the effects between endophyte × cultivar observed in one season may not be consistently seen through the duration of an individual trial.

The P209WOD trial is an example of site variation affecting an outcome on yield performance and the potential for recovery post stress (Tables 2 and 5). The confounding interaction reported in this data is the significant DM yield advantage in the third summer of AR1 over AR37 (Table 5). This trial was reported by Popay et al. (2012) for porina damage; AR37 plots had significantly lower porina feeding damage and higher surviving plant densities post the second autumn than AR1 and E- plots, which were not different from each other. Surprisingly, the damage caused by porina did not result in a DM yield advantage for plots with AR37 over AR1 in the third summer, highlighting the ability of plants to recover, and mitigate yield production benefits. During the porina assessment in July 2011, plant density was scored on a 1-5 scale (5=high), with ONE^{50} AR37 scoring 4.75 which was significantly greater than ONE^{50} AR1 which was scored a 2.25 (Popay et al. 2012). This highlights that even with approximately 50% fewer plants, the remaining plants with AR1 were able to outperform AR37 the following summer (Table 5). Factors contributing were not verified but general climatic conditions, trial fertiliser, weed control and grazing management provided opportunity for compensatory growth in the AR1 plots. It has been shown that it is very often the combined pressures of insect pests and drought that prove detrimental to ryegrass persistence (Hewitt et al. 2021). This result may not have been seen under farm management, where further pressure could be applied through grazing management, invasion by weed species or if the insect damage had occurred during a dry period. For on farm, it is best to consider the most appropriate cultivar × endophyte combination that allows the greatest resilience to pasture productivity under different biotic and abiotic stressors that the site is at risk of experiencing.

Outcomes

This study confirms the importance of *Epichloë* endophyte in New Zealand perennial ryegrass pastures for maintaining ongoing DM production. It is unknown exactly where or when a detrimental insect incursion affecting plant persistence will occur across and within regions of New Zealand, thus from a practical recommended point of view, the use of the endophyte that conveys the broadest level of insect protection for the farm system and livestock class seems applicable. Based on the endophytes in the current study, AR37 relative to AR1 and E- offers an opportunity for farmers to mitigate potential risks from a larger number of ryegrass insect pests across New Zealand.

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