Epichloë endophytes – new perspectives on a key ingredient for resilient perennial grass pastures

John R. CARADUS[1,*], David F. CHAPMAN[2], Tim COOKSON[3], Blair COTCHING[4], Matthew H. DEIGHTON[3], Liam DONNELLY[5], Justine FERGUSON[6], Sarah C. FINCH[9], Sarah GARD[7], David E. HUME[8], Linda J. JOHNSON[8], Graham A. KERR[4], Michael G. NORRISS[6], Kate PEDDIE[7] and Alison J. POPAY[9]

[1] *Grasslanz Technology Ltd, PB 11008, Palmerston North 4442, New Zealand*
[2] *DairyNZ, PO Box 85066, Lincoln University, Canterbury 7647, New Zealand*
[3] *Cropmark Seeds Ltd, 49 Manion Road, Rolleston 7677, New Zealand*
[4] *Barenbrug NZ Ltd, 2547 Old West Coast Road, RD1, Christchurch 7671, New Zealand*
[5] *Seed Force Ltd, PO Box 16-625, Hornby South, Christchurch 8411, New Zealand*
[6] *PGG Wrightson Seeds Ltd, PO Box 69175 Lincoln, Christchurch 7640, New Zealand*
[7] *Germinal New Zealand Ltd, 144 Tancred Street, Ashburton 7700, New Zealand*
[8] *AgResearch Ltd, PB 11008, Palmerston North 4442, New Zealand*
[9] *AgResearch Ltd, PB 3123, Hamilton 3240, New Zealand*
*Corresponding author: john.caradus@grasslanz.com

Highlights
The confirmation that *Epichloë* endophytes are important for pest resistance in New Zealand pastures led to the development of a range of novel endophyte strain × host plant combinations that enhance the persistence of the grass, while mitigating adverse effects on grazing animals. Successfully delivering these endophytes to the pastoral industry has required the development of a range of scientific and commercial capabilities. In March 2012, the New Zealand proprietary seed industry established the Endophyte Technical Committee. This is a cooperative forum to ensure that endophyte strains in commerce or under development are tested uniformly, and to publish industry-agreed descriptions of the animal safety and insect control of commercial endophyte strains each year.

Keywords: animal health and welfare, biotic resistance, commercialisation, mutualism, testing protocols

Background
Plants and microbes have long been recognised to co-exist naturally, and in some cases benefit their mutualistic, symbiotic partner (Read 1994). *Epichloë* fungal endophytes form a mutualistic association with some temperate grass species that is essential for resilient pastures in New Zealand (Hume et al. 2020). These fungal endophytes are more than a biological curiosity; they have a significant economic impact on pastoral farming in many temperate areas of the world. This impact can be both positive in providing biotic and abiotic resistance/tolerance, and in some cases negative with the potential to cause animal health and welfare effects for grazing animals. In the USA, fescue toxicosis caused by *Epichloë* endophyte in tall fescue (*Festuca arundinacea*) has been estimated to have a US$1B per year cost (Comis 2000; Aitken & Strickland 2013). In New Zealand, ryegrass (*Lolium perenne*) staggers caused by the standard (or wild-type) strain of endophyte reduced gross financial return by 16% relative to an endophyte-free ryegrass grazing system at Lincoln (Fletcher 1999), and has been estimated to cost up to NZ$100M per year before selected endophytes were used (Anon 2007). In Australia, ryegrass toxicosis caused by endophyte was estimated to cost A$100M per year (Leury et al. 2014). These positive and negative consequences result from the secondary metabolites or alkaloids produced by the fungus when hosted within the grass plant (Kuldau & Bacon 2008).

The challenge faced by researchers in New Zealand, since the discovery of *Epichloë* endophytes in the early 1980s, has been to identify strains that provide more of the benefits (e.g., improved persistence through increased control of insect pests) and minimise the negative impacts (e.g., ryegrass staggers, heat stress, and production losses, Easton 1999). The science that has provided an understanding of the biology of endophytes and their role in pastures, has led to the delivery of a number of selected endophyte strains that are or have the potential to revolutionise the pastoral industry (Johnson & Caradus 2019).

This review summarises recent advances in understanding the biology of *Epichloë* endophytes, describes the process of identifying efficacious strains and their delivery to the market with industry-agreed performance ratings, and outlines future directions for research and development.

Epichloë biology and chemistry
The importance of *Epichloë* endophytes in pastoral agriculture was first identified in the late 1970s in the USA (Bacon et al. 1977) and in the early 1980s in New

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Zealand (Fletcher & Harvey 1981) through associating their presence with reduced animal health when grazing tall fescue and ryegrass, respectively. These health issues resulted from livestock ingestion of alkaloids produced by the endophyte growing within the host grass.

Asexual strains of *Epichloë* endophytes are noted for their obligate mutualism, meaning that they have no free-living form in nature and are transmitted vertically within the seed (Card et al. 2016). They provide significant benefit to the host plant while the plant provides essential nutrients and a moisture-laden environment for the fungus. The fungus has adapted to growing between the cells of leaf tissue (absent from the root system) such that hyphae attached to the wall of leaf cells grow through intercalary expansion (Christensen et al. 2008; Voisey 2010) rather than the apical growth that occurs for most other fungi (Bartnicki-Garcia & Lippman 1972).

It is the alkaloids produced by *Epichloë* endophytes that makes them so important. The biochemical pathways and genes involved in the production are complex but our understanding is increasing (Schardl et al. 2013). The four major alkaloid classes are the indole diterpenes (lolitrem B, paxilline, epoxyjanthitrems and terpendoles), the ergot alkaloids (ergovaline and its analogues), lolines (1-aminopyrrolizidines), and peramine (pyrrolopyrazines). Particular alkaloids have known bioactivity against mammals (Table 1a) and/or invertebrates (Table 1b). For each invertebrate, testing is required at both the adult and larval stages. Examples are the effects of the selected endophyte strain AR37 on root aphid (*Aploneura lentisci*), Argentine stem weevil (ASW, *Listronotus bonariensis*) larvae and African black beetle (*Heteronychus arator*) adults (Popay & Gerard 2007; Popay & Thom 2009; Popay & Cox 2016; Popay et al. 2021), and various endophytes on pasture mealybug (*Balanococcus poae*, Pennell et al. 2005). In addition, the concentration of alkaloids in the parts of the plants eaten by an insect strongly influences the level of bioactivity of an endophyte strain. Avoidance of strains that produce lolitrem B, some of the terpendoles, and high levels of ergovaline, paxilline and epoxyjanthitrems will reduce animal health and welfare issues in grazing animals. Whether a particular endophyte strain will affect animals, or the severity of that effect, is highly variable depending on the situation and management, and is related to the alkaloid intake of animals, usually calculated as mg/kg $LW^{0.75}$/day (Nicol & Klotz 2016). In contrast, an increasing expression of peramine or loline compounds is likely to promote improved resistance to plant insect pests with no negative impact on animals.

Table 1 Bioactivity of known chemistry produced by *Epichloë* endophytes on (a) mammals and (b) invertebrates.

(a) Mammals

***Epichloë* associated chemistry**	**Potential impact on mammals**	**Reference**
Ergot alkaloids		
Ergovaline	Fescue toxicosis: inability to regulate body temperature resulting in heat or cold stress	Tor-Agbidye et al. 2001
	Feed deterrent: reduced liveweight gains	Panaccione et al. 2006
Chanoclavine	Not toxic at levels found in grasses	Finch et al. 2019
Indole-diterpene alkaloids		
Lolitrems A, B and F	Tremorgens that affect muscular coordination. Lolitrem B causes ryegrass staggers	Miles et al. 1992; Munday-Finch et al. 1995, 1996; Guerre 2016
Terpendole C and M	Mild tremorgen: generally short-lived in mice when injected	Munday-Finch et al. 1997; Gatenby et al. 1999
Epoxyjanthitrems	Tremorgen: less potent than lolitrem B and staggers induced by these compounds are generally less severe and of shorter duration	Fletcher & Sutherland 2009; Babu et al. 2018
Paxilline	Moderate tremorgen: fast acting but short-lived in mouse bioassay; marked increases in respiration rate, heart rate and blood pressure when administered intravenously	McLeay & Smith 1999
Pyrrolopyrazine alkaloid		
Peramine	No known mammalian toxicity	Pownall et al. 1995
Pyrrolizidine alkaloids		
N-acetyl loline (NAL)	No known mammalian toxicity	Schardl et al. 2007; Finch et al. 2017
N-formyl loline (NFL)	No known mammalian toxicity	Schardl et al. 2007; Finch et al. 2017
N- acetylnorloline (NANL)	No consistent mammalian toxicity	Schardl et al. 2007; Finch et al. 2017

(b) Invertebrates

***Epichloë* associated chemistry**	**Impact on invertebrates**	**Reference**
Ergot alkaloids		
Ergovaline	African black beetle adults: antifeedant	Popay & Ball 1998
	Argentine stem weevil adults: feeding deterrent	Popay et al. 1990
	Fall armyworm larvae: deterrent	Clay & Cheplick 1989
	Japanese beetle larvae: deterrent	Patterson et al. 1991
	Large milkweed bug: deterrent	Yates et al. 1989
	Lesion nematode: attractant but toxic	Bacetty et al. 2009
	Deroceras molluscs: stimulates feeding	Barker 2008
Chanoclavine	African black beetle: no effect	Hudson et al. 2021
Clavines	Grass grub larvae: deters feeding – tentative result	Popay & Tapper 2007
Indole-diterpene alkaloids		
Lolitrem B	Argentine stem weevil larvae: feeding deterrent and toxin	Popay et al. 2003a
	African black beetle adults: no effect	Ball et al. 1997
Epoxyjanthitrems	Porina larvae: reduced feeding and survival	Popay et al. 2012; Hennessy et al. 2016; Babu et al. 2018; Finch et al. 2020
Paxilline	Argentine stem weevil larvae: feeding deterrent	Rowan 1993
	Porina larvae: reduced feeding and weight gain	Babu 2008
	Deroceras slug: attractant	Barker 2008
	African black beetle adults: no effect	Ball et al. 1997
Pyrrolopyrazine alkaloid		
Peramine	Argentine stem weevil adults and larvae: deters oviposition	Rowan et al. 1990
	Root aphid: no effect	Popay & Gerard 2007
	African black beetle: no effect	Ball et al. 1997
Pyrrolizidine alkaloids		
Lolines – not defined	Grass grub larvae: reduced root feeding and larval weight gain; deterrent effect	Popay & Lane 2000; Popay et al. 2003b; Patchett et al. 2008b, 2011
	African black beetle: antifeeding effect on larval and adult stages	Bryant et al. 2010; Barker et al. 2015a
	Argentine stem weevil: feeding deterrent and death of larvae; reduces oviposition	Patchett et al. 2008a; Jensen et al. 2009; Barker et al. 2015c
	Aphids: feeding deterrent and toxic	Wilkinson et al. 2000; Bultman et al. 2006
	Black field cricket: deterrent	Barker et al. 2015b
	Porina larvae: reduced feeding and weight gain	Popay & Lane 2000
	Root aphid: reduced populations	Schmidt 1993
	Fall armyworm: deterrent	Riedel et al. 1991
	European corn borer: deterrent	Riedel et al. 1991
	Deroceras slug: reduced feeding	Barker 2008
N-acetyl loline (NAL)	Grass grub: strong deterrent	Popay & Tapper 2007
N-formyl loline (NFL)	Argentine stem weevil larvae: increased mortality	Jensen et al. 2009
	Grass grub larvae: strong deterrent	Popay & Lane 2000; Popay & Tapper 2007
	Lesion nematode: repellent and toxic	Kimmons et al. 1990; Bacetty et al. 2009
N-acetylnorloline (NANL)	Argentine stem weevil larvae: increased mortality	Jensen et al. 2009
	Grass grub larvae: weak deterrent	Popay & Tapper 2007

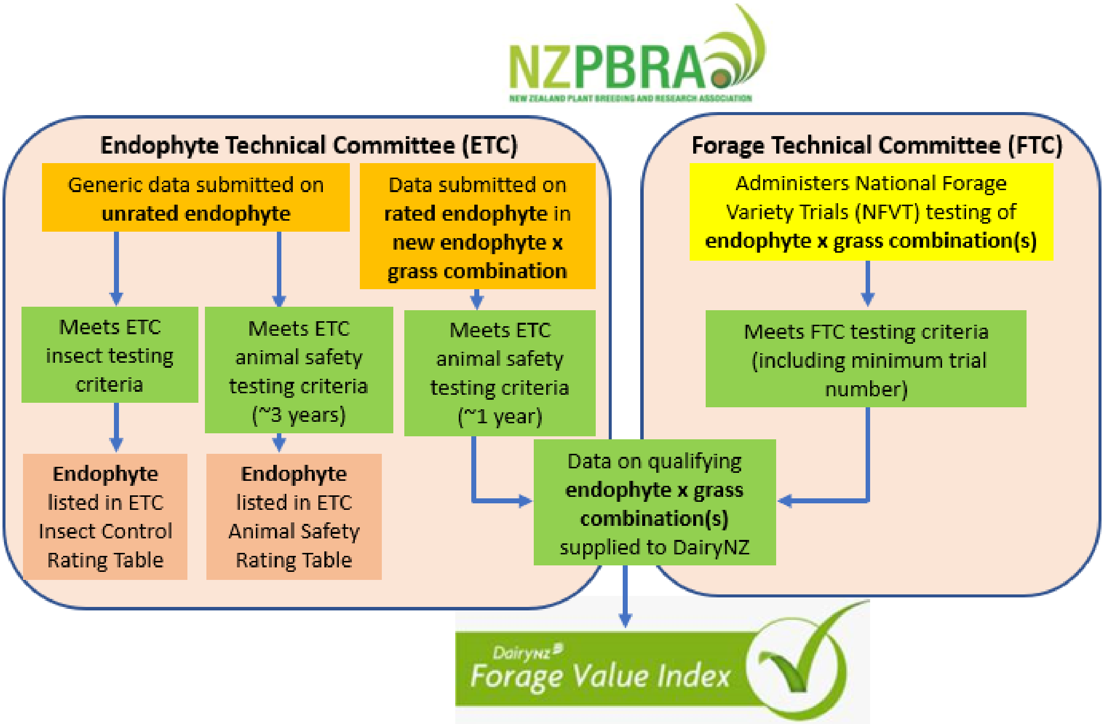

Figure 1 The process for assessing new endophytes or new cultivar × endophyte combinations within the New Zealand Plant Breeding and Research Association system and attaining eligibility for the DairyNZ Forage Value Index.

Development of efficacious *Epichloë* strains

Up to 1990 the standard strain of endophyte was widespread throughout New Zealand pastures, believed to have been imported inadvertently in ryegrass seed from Europe, particularly the United Kingdom (van Zijll de Jong et al. 2008). While it is likely only some of this seed contained endophyte, high levels of standard endophyte in ryegrass pastures would have been selected for by the presence of several insect pests compounded by summer moisture deficits. Once the toxicity of standard endophyte was understood, solutions to this dilemma were sought (Easton et al. 2001).

Commercial delivery of *Epichloë* strains

The industry Endophyte Technical Committee (ETC) was established in March 2012, and operates under the auspices of the New Zealand Plant Breeding and Research Association (NZPBRA), with the purpose of providing industry-agreed, scientifically-based ratings for the effectiveness of endophytes against insect pests, and the effects of endophytes on animal health and performance. These objectives have been achieved by participant companies voluntarily adopting a standard set of testing protocols held and managed by the ETC.

The ETC recognise two levels of testing: 1) a new endophyte entering the system for the first time; and 2) an endophyte tested previously within a new grass cultivar (i.e., a new 'cultivar × endophyte combination'). For new endophytes, the testing requirements are more rigorous (Figure 1).

Insect testing

For a totally new endophyte to be rated for its insect control by the ETC, it must go through the full testing schedule outlined for the relevant individual insect pest. For a new cultivar × endophyte combination, where the endophyte has been rated previously, it is necessary to have only field trial data from one trial where the minimum insect damage levels described in the relevant protocol occur in the nil endophyte controls. Testing protocols have been developed for Argentine stem weevil, African black beetle, grass grub (*Costelytra giveni*), pasture mealybug, porina (*Wiseana cervinata*), and root aphid. New endophytes are tested under controlled conditions against both positive (i.e., endophytes known to have a strong effect on a particular insect) and negative (endophyte-free) controls. Endophyte status of plants is expected to be 100% infected or endophyte-free in no-choice insect experiments. Depending on the insect, numbers are determined at the completion of the trial, egg laying counted and/or levels of damage or feeding are evaluated (e.g., classed as minor, moderate or severe).

Animal testing

The ETC protocols and processes ensure relevant animal health data on endophytes are generated before a new endophyte is released commercially for consumption by livestock on New Zealand farms.

For animal performance and safety, the presumption is that for a totally new endophyte, the technology developer will undertake in-depth testing before releasing to market, because there is a commercial imperative to do so. For the ETC to provide a rating for a new endophyte, a minimum of 3 years of trials is required, with measurements collected in several seasons each year, that ensures animals are exposed to a safety challenge as confirmed by the standard endophyte control. For a previously untested cultivar × endophyte combination, where the endophyte has a safety rating already, the requirement for ETC rating is for 1 year of testing where results must agree with previous testing of the same endophyte strain.

The ETC protocols have been developed and modified over time as research has identified the extent of the responses in grazing animals consuming a sufficient amount of alkaloid from grass containing endophytes to develop toxicity (Thom et al. 2012). The animal safety trial for evaluation of candidate endophytes and cultivar × endophyte combinations comprises at least three treatments: 1) the candidate endophyte; 2) a standard endophyte control; and 3) a safe (e.g. selected endophyte strain AR1) endophyte control. The management conditions in the protocols are set so that testing is a worst-case scenario for toxicity, where animals are exposed to a high level of endophyte alkaloid intake. Animal welfare is a high priority in these evaluations and all trials are subject to animal ethics approval, via an application to an Animal Ethics Committee, which operates under the Animal Protection (code of ethical conduct) Regulations Act, 1987.

The worst-case scenario conditions for testing involves small paddocks sown without clover, and with non-sown plant species controlled as necessary through herbicide application(s). Treatments are sown in separate paddocks with a minimum of three replicates. Endophytic pastures must have >85% of grass tillers infected with the appropriate endophyte strain. The pastures are grown under moderate moisture deficit to elevate alkaloid production. In the paddocks a large bulk of feed (≥4000 kg dry matter (DM)/ha) is left to accumulate over early to mid-summer, and the trial period starts with weaned lambs introduced to paddocks from late summer to early autumn and continues for 42 days. Lambs are monitored daily and any differences between those grazing the test cultivar × endophyte combination and controls are noted. When signs of ryegrass staggers are first seen, sheep are scored twice per week for the degree of ryegrass staggers using the Keogh scoring system (Keogh 1973). For a trial to be valid, the ryegrass staggers observed in sheep grazing the standard endophyte and the safe controls must differ, and the staggers of stock grazing the standard endophyte control must reach an average score of 2.3 on the Keogh scale.

In addition, response of lambs to heat stress is determined through recording respiration rates and body temperatures under warm, humid conditions. Animal weight gain is also recorded but data interpretation can be difficult as the trials are managed for a worst-case endophyte toxicity and not for optimal animal growth. The key comparison is between the treatment groups grazing the cultivar containing the candidate endophyte, and those grazing the safe control endophyte.

While a wide range of grazing animals can suffer from endophyte toxicity, most New Zealand research has focused on sheep as the model ruminant. Sheep are not an ideal model for milk production in dairy cows (Thom et al. 2012), but on-farm experience and testing with dairy cows over the past 30-40 years has largely validated the use of young sheep as an appropriate model for ruminants. Other grazing animals such as horses, deer and alpaca may have different responses to endophyte alkaloids so data generated from sheep cannot be used to predict outcomes in these species.

In specific situations, endophytes can transition from an animal grazing test to a chemistry-only test for animal safety rating by the ETC. For example, this has happened for the AR1 strain, when the animal toxicity risk profile has been well established through numerous grazing trials and on-farm use, and the endophyte chemistry effects on animals are understood well. As with the grazing trials, chemistry-only testing uses worst-case scenario conditions, whereby field plots under moderate drought stress are sampled from late summer to early autumn and analysed for the required endophyte alkaloids. Data for the test cultivar × endophyte combination are compared against a cultivar tested previously with the candidate endophyte to ensure that the new endophyte-infected cultivar has the expected alkaloid concentrations based on previous datasets. If outside the expected range, the new cultivar × endophyte combination may need to be evaluated in a grazing safety trial before commercial release.

Commercialised endophyte strains

The first major commercial release of a selected endophyte in New Zealand was in 2001 when AR1 was marketed to combat ASW and remove the threat of ryegrass staggers (Popay et al. 1999). Since then, several other strains have been released with differing chemistry and impacts (Table 2). The uptake of endophyte technologies by New Zealand farmers has been high with an estimated 90% of proprietary ryegrass now containing a selected endophyte strain. The industry uptake of AR1 was rapid with about 70% of proprietary perennial ryegrass seed sold containing AR1 only 6 years from first sale (Caradus et al. 2013). The brand NEA2 endophyte was released initially in ryegrass cultivar 'Tolosa' in 2001, but had a limited life due to its low seed production. Since 2008, AR37, the brand NEA2 and AR1 have, and still form, the bulk of the market for selected endophytes. While actual alkaloid levels (ppm or mg/kgDM) can vary due to environment and time of year, the relative levels between strains and their ranking are consistent.

Commercial endophyte strain ratings

Industry representatives on the ETC have agreed on ratings for the level of control provided by commercialised endophyte strains of the most prevalent New Zealand pasture pests (Table 3a). New endophyte strains and species × endophyte strain combinations are tested against these insect pests using protocols as discussed previously, with this information added to Table 3a. These ratings vary depending on the type of grass host (e.g., Italian or perennial ryegrass) and ploidy for ASW, as these can impact the expression of compounds and the attractiveness of the host to the insects. However, it is the endophyte strain that determines which compounds are expressed (Easton et al. 2002).

Similarly, the ETC produce industry-agreed animal safety tables (Table 3b), based on the results of the animal safety trialling using protocols designed to expose animals to worst-case management, that is not normal farm practice, which is why an explanation is given above the ratings table. The type of information required for a new cultivar × endophyte combination to meet the animal safety criteria is shown in Table 5 with these types of results forming the basis for establishing the endophyte animal health ratings.

NZPBRA member companies have agreed with DairyNZ that any new endophytes, or existing endophytes in a new host ryegrass, must be tested for animal safety and have an agreed rating from the ETC to be eligible for listing in the co-operative NZPBRA-DairyNZ Forage Value Index (FVI, Figure 1). The ETC animal safety table (Table 3b) provides information on possible negative impacts of endophyte strains that are not captured by the current FVI assessments. Yield trials conducted in the National Forage Variety Trial system under the auspices of the NZPBRA, which provide the base yield data for the FVI, are not monitored formally

Table 2 Description of current (2020) commercial *Epichloë* endophyte strains in New Zealand.

Grass species	Brand (endophyte strain(s) if different from brand name)	Year of commercial release in New Zealand	Known alkaloids	Reference
Ryegrass	AR1	2001	High peramine	Fletcher 1999; Tapper & Latch 1999
	NEA2 (mix of NEA2 and NEA6)	2001	Medium ergovaline, medium-low peramine, very low lolitrem B	Logan et al. 2015; Eady et al. 2017; Fletcher et al. 2017; Hewitt et al. 2020; Popay et al. 2021
	AR37	2007	High epoxyjanthitrems	Tapper & Lane 2004; Finch et al. 2020
	NEA (NEA2)	2012	Low ergovaline and peramine, very low lolitrem B	Stewart et al. 2014; Logan et al. 2015
	Edge	2015	Medium peramine	Kitson 2017; Pers. comm. WJ Mace, AgResearch
	NEA4 (mix of NEA2 and NEA3)	2016	Medium ergovaline, medium-low peramine, very low lolitrem B	Logan et al. 2015
	Happe	2016	Loline	Dairy Australia 2019
Festulolium	U2	2013	NFL[1] (68% of total lolines), followed by NAL[1] (23%), and NANL[1] (8%)	Nboyine et al. 2017
Tall fescue	MaxP (AR542)	2003	NANL[1], peramine	Popay & Tapper 2007
	MaxP (AR584)	2011	NFL, NAL, NANL[1], peramine	Popay & Tapper 2007
Meadow fescue	MaxR (AR1017)	2020	NFL, NAL, NANL[1]	Pers. comm. WJ Mace, AgResearch

[1] NAL (N-acetyl loline); NANL (N-acetylnorloline); NFL (N-formyl loline)

for insect infestation, nor are they necessarily located in areas or undertaken in years where there is a high insect pest challenge, so the DM yields do not always reflect differences due to the effects of endophyte. Thus farmers should consider the ETC insect ratings alongside the FVI, with knowledge and experience of the likely insect pests causing damage on their farm. Endophyte strain choice can make a major difference to pasture resilience.

Future opportunities for *Epichloë* research and development

Epichloë endophytes will continue to be essential for resilience of many temperate grass pastures. However,

Table 3a Industry Endophyte Technical Committee ratings of commercialised endophyte strains for insect control (approved by NZPBRA Executive 22nd September 2020).

Endophyte brand	Argentine stem weevil	Pasture mealy bug	Black beetle	Root aphid	Porina	Grass grub	Field cricket
Diploid perennial ryegrass							
AR1	++++	++++	+	-[2]	-	-	Not tested
NEA2	+++	(++++)	+++	++	Not tested	-	Not tested
NEA4	+++	(++++)	+++	++	Not tested	Not tested	Not tested
AR37	++++[1]	++++	+++	++++	+++	+	Not tested
Standard endophyte	++++	++++	+++	++	+	-	Not tested
Without endophyte	-	-	-	-	-	-	Not tested
Tetraploid perennial ryegrass							
AR1	(+++)	(++++)	+	-[2]	-	-	Not tested
AR37	(+++)[1]	(++++)	+++	++++	(+++)	+	Not tested
Without endophyte	-	-	-	-	-	-	Not tested
Italian and short term (hybrid) ryegrass							
AR1	++	(++++)	+	-[2]	Not tested	-	Not tested
NEA	Not tested	(++++)	+++	Not tested	Not tested	-	Not tested
AR37	+++[1]	(++++)	+++	Not tested	Not tested	-	Not tested
Without endophyte	-	-	-	-	-	-	Not tested
Festulolium							
U2	++++	(++++)	++++[3]	++++	(++)	+++	+++
Continental tall fescue							
MaxP (AR584)	Not tested	Not tested	+++	(++++)	Not tested	(++)	+++
Without endophyte	-	-	-	-	-	-	-

- No control.
+ Low level control: Endophyte may provide a measurable effect but is unlikely to give any practical control.
++ Moderate control: Endophyte may provide some practical protection, with a low to moderate reduction in insect population.
+++ Good control: Endophyte markedly reduces insect damage under low to moderate insect pressures. Damage may still occur when insect pressure is high.
++++ Very good control: Endophyte consistently reduces insect populations and keeps pasture damage to low levels, even under high insect pressure.
( ) Provisional result: Further results needed to support the rating. Testing is ongoing.
[1] AR37 endophyte controls Argentine stem weevil larvae, but not adults. While larvae cause most damage to pastures, adults can damage emerging grass seedlings. In Argentine stem weevil prone areas it is recommended to use treated seed for all cultivars with novel endophyte.
[2] AR1 plants are more susceptible to root aphid than plants without endophyte.
[3] Active against black beetle adults and larvae.

seeking continual improvement through the discovery of new, naturally-occurring endophyte strains has become difficult due to limited natural genetic variation that exists. This has led researchers to utilise genetic technologies such as the CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-related nuclease 9) system (Shi et al. 2017). To date, this technology is the most versatile genomic engineering tool with an unprecedented level of precision and control in gene editing that does not require insertion of foreign DNA. As endophyte strains exhibit limited variation at the genetic level,

Table 3b Endophyte animal safety: Ryegrass, *festulolium* and continental tall fescue (approved by NZPBRA Executive 22nd September 2020).

The information in this table is based on animal safety trialling protocols designed to expose animals to simulated worst-case scenario management. This involves forcing them to graze deep into the base of pure perennial ryegrass pastures that have been allowed to grow for several weeks over late spring/summer (similar to a hay crop) where they will encounter the highest concentrations of harmful endophyte chemicals if these are present.

This management does not represent normal farm practice although similar situations may arise on farms in rare circumstances. Under normal farm grazing practices, the contribution of basal pasture material to total animal dry matter intake is relatively low and therefore the intake of harmful chemicals (if they are present) is diluted. Thus, the likelihood of adverse effects on animals is reduced, but the potential for problems to occur may still exist if the endophyte brand is rated <4-star for 'freedom from staggers' and/or there are comments on animal performance which flag potential issues.

Comments on animal performance have been moderated based on information from other trials (in addition to the formal animal safety testing protocols), consideration of the 'normal' grazing management practices implemented on farm (see previous paragraph), and recognition that animal diets are very seldom pure ryegrass. Other dietary components such as clovers or non-ryegrass grass species, crops or supplements will dilute the intake of endophyte alkaloids.

Endophyte brand	Freedom from staggers		Effects on animal performance
	Sheep and lambs	Cattle and dairy cows	
AR1	++++	++++	High level of animal performance
AR37	+++	++++	Typically provides a high level of animal performance. Can cause ryegrass staggers in sheep and lambs in extreme circumstances. Lamb liveweight gain can be reduced during periods of severe staggers. While ryegrass staggers has never been observed in cattle and dairy cows, it could occur on rare occasions.
NEA	++++	++++	High level of animal performance
NEA2	++++	++++	Typically provides a high level of animal performance. Lamb liveweight gain could be reduced in extreme circumstances. While no effects have been observed in cattle and dairy cows, body temperature could be elevated on rare occasions.
NEA4	++++	++++	Typically provides a high level of animal performance. Lamb liveweight gain could be reduced in extreme circumstances. While no effects have been observed in cattle and dairy cows, body temperature could be elevated on rare occasions.
U2	++++	++++	High level of animal performance
MaxP (AR584)	++++	++++	High level of animal performance
Standard endophyte	+	++	Can cause ryegrass staggers in sheep and lambs, and significantly decrease lamb growth rates in summer and autumn, and significantly increase dags and heat stress In dairy cows, it has been shown to depress milksolids production through summer and autumn.
Without endophyte	++++	++++	High level of animal performance

Key to ryegrass staggers ratings:
+ Likely to cause severe staggers in most years
++ Can cause severe staggers in some years
+++ Can cause severe staggers occasionally
++++ Very unlikely to cause staggers

endophytes could be designed to express desirable combinations of chemistry required for protecting the host plant against insect pests, without livestock toxins. In New Zealand, all forms of gene editing are currently classified as genetic modifications and are highly regulated. However, if New Zealand regulatory law was modified to facilitate the commercialisation of gene-edited endophyte products, this technology could significantly improve pasture resilience. Of note, in some countries (e.g., Australia, USA) certain types of CRISPR technologies are not regulated as genetic modifications (Waltz 2016; Friedrichs et al. 2019), likely easing the commercialisation of gene-edited endophytes in agriculture around the world.

The experience of plant breeders matching endophytes to ryegrass germplasm is only recent, and it is likely that good gains will be made, particularly with a new revolution of phenotyping and genotyping techniques underway. Discovering what endophyte(s) 'fit' best with which ryegrass will improve the symbiotic effect, leading to increased yield and pasture resilience. Other advantages could be better endophyte transmission in seed production, and improved longevity of endophyte in seed storage. Greater understanding of alkaloid production and pathways will allow greater control of alkaloid levels within pastures, potentially increasing or decreasing these for a grass cultivar as required.

Conclusions/future implications

Epichloë endophytes are crucial for grass persistence in much of New Zealand, helping protect them from a range of insect pests, thereby delivering robustness or resilience particularly in times of moisture deficit. The sustainable way the natural grass-endophyte provides this, means endophytes have a strong future, particularly if the trend for international consumers of New Zealand pastoral produce continues to demand less use of synthetic chemicals, such as pesticides.

The ryegrass–*Epichloë* mutualism was first discovered in New Zealand due to its association with animal health disorders of staggers and later heat stress, amongst others. New Zealand researchers have led the world in identifying strains of *Epichloë* that while providing resistance to insect pests, do so with minimal or no animal health and welfare concerns. It is now the exception for ryegrasses to be sown in New Zealand without a selected endophyte. The benefits afforded by *Epichloë* endophytes are due to a range of secondary metabolites that they produce. Much is known about the mode of action and effects of a number of these. However, there is still much to learn and progress to be made about many unknown compounds, and the benefits or downsides that may be associated with them.

The proprietary seed industry has established the ETC with several key purposes. Firstly, to coordinate

Table 4 Results on the performance of AR37 compared with nil endophyte, and standard endophyte against a range of insect pests found in New Zealand pastures (Pennell et al. 2005; Popay et al. 2008; Thom et al. 2014).

Treatment	Argentine stem weevil tiller damage (%)	African black beetle (no./m^2)	Porina tiller damage (%)	Pasture mealybug (no. per core)	Root aphid (no. per plant)	Grass grub (no. grubs/m^2)
AR37	2 a	23 a	14 a	0.3 a	2 a	40 a
Standard	3 a	17 a	29 b	0.6 a	171 b	108 a
Nil endophyte	26 b	64 b	35 b	18.8 b	244 b	55 a *

Within columns, numbers with different letters differ at $P<0.05$
* Number was low due to prior black beetle larval damage resulting in poor survival of nil pastures and no plant material for grass grubs to eat.

Table 5 Average daily weight gain (ADG), ryegrass staggers score on day 28 (from start of grazing) (using the Keogh scoring system), mean respiration rate, and rectal temperatures of lambs grazing a candidate cultivar with AR37, AR1, AR37 and standard endophyte in a common ryegrass cultivar.

Treatment	Endophyte infected tillers (%)	ADG (g/day)	Ryegrass staggers score	Mean respiration rate (breaths/min)	Mean rectal temperature (°C)	Epoxy-janthitrem level (ppm)
Candidate cultivar AR37	89 a	52 a	0.9 a	163 a	40.95 a	52 b
GA66 AR1	95 a	49 a	0.4 a	161 a	40.89 a	0 a
GA66 AR37	80 a	61 a	0.5 a	164 a	40.78 a	46 b
GA66 Standard	88 a	NA	>4.0 b	182 b	41.19 b	0 a

[a] Within columns, numbers with different letters differ at $P<0.05$
NA = not available as lambs suffered severe staggers and had to be removed from the trial to meet animal ethics requirements

and improve the testing of *Epichloë* strains using agreed protocols for animal safety and insect control. Secondly, to assess information on new endophytes and new endophyte × cultivar combinations as to their performance. Lastly, and arguably most importantly, to provide clear, industry-agreed information to the farming industry about how endophytes perform.

The future identification and delivery of even more beneficial *Epichloë* endophytes will be reliant on further discovery of new strain variants in germplasm collections, improved genomic understanding on how to improve compatibility between strain and host plant genetics, and/or the acceptance and deregulation of gene editing technologies.

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